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Effect of vermicompost, vermicompost extract and vermiwash on growth, yield and antimicrobial activity of *Andrographis paniculata*

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ABSTRACT

Andrographis paniculata is an important medicinal herb used to treat various diseases in human beings. The objective of the present study was to investigate the effect of vermicompost and its related products on growth, yield and antimicrobial activity of A. paniculata. The experiment was conducted in a completely randomized block design (CRBD) with six treatments using different combinations of vermicompost (VC), vermicompost extract (VCE), vermiwash (VW), recommended dose of fertilizer (RDF) and one control. The present study clearly revealed that the combined application of VC, VCE and VW significantly improves the growth, yield and antimicrobial activity of A. paniculata than RDF.

Keywords: Vermicompost, vermicompost extract, vermiwash, antimicrobial activity, *A. paniculata*.

INTRODUCTION

Andrographis paniculata (Acanthaceae) is one of the medicinally important herbal plant with extreme bitterness and widely distributed throughout the plains of India (Uttar Pradesh, Assam, Madhya Pradesh, Tamil Nadu and Kerala). In general, the leaves and roots were used in traditional Siddha and Ayurvedic medicines as well as tribal medicines in India and some other countries for multiple clinical applications. *A. paniculata* is being used mainly for treating common cold, fever, liver diseases, diabetes and snake bite [1]. Extensive research has revealed that *A. paniculata* has a broad range of pharmacological effects such as hepatoprotective [2], antimalarial [3], antidiarrhoeal [4], anti-inflammatory [5, 6], antibacterial and antiviral [7] properties.

Vermicompost is the humus rich dark brown organic product obtained during the vermicomposting process which involves physical fragmentation and biochemical oxidation of organic wastes by the joint action of earthworms and its associated microorganisms [8-11]. Now-a-days vermicomposting process are gaining momentum in sustainable agriculture not only as an organic fertilizer but also providing certain value added products like vermiwash, humic acid, vermicompost extract and vermi meal. Vermicompost is excellent organic manure that contains higher level of organic matter, organic carbon, total and available NPK and micronutrients, beneficial microbes and enzyme activities [11, 12].

The present study was aimed to find out the effect of application of vermicompost, vermicompost extract and vermiwash on the growth, yield and antimicrobial activities of methanolic extract of leaf, stem and root of *A. paniculata* against some potential human pathogens.

MATERIALS AND METHODS

Vermicompost, vermicompost extract and vermiwash: Vermicompost used in the study were obtained from poly culturing of *E. eugeniae, E. foetida* and *P. excavatus* with cow dung as the substrate. Their physico-chemical properties were pH - 7.06; EC - 1.68 dSm^{-1}; Nitrogen - 1.86%; Phosphorus - 1.12%; Potassium - 1.80%; organic carbon 29.82% and C:N ratio 16.03. Vermicompost extract was prepared according to [13]. Vermicompost was mixed with tap water at a ratio of 1:2 (v/v) and storing this mixture at room temperature for about 24 h and filtered through Whatman No. 2 filter paper. Vermiwash was prepared by using the method of [14]. About 100 g of adult earthworms were collected and released into a trough containing 50 ml of Luke warm water (37-40°C) and agitated gently for two minutes. The agitation in the Luke warm water makes the earthworms to release sufficient quantity of body fluid and mucus. Earthworms are then taken out and again released back into the trough containing 50 ml of distilled water (room temperature) to wash the mucus sticking to their body surface and this also helps the earthworms to revive from the shock. The collected vermiwash is diluted with water in the ratio 1:10 before spraying. The vermicompost extract and vermiwash were sprayed at 15 days interval period.

Experimental design: A pot culture study was conducted in 45 × 30 × 30 cm rectangular cement pots with completely randomized block design (CRBD) and triplicates were maintained. The treatments were T_1 - Control, T_2 - RDF (NPK @ 30:30:20 kg ac^{-1}), T_3 - VC @ 2 t ac^{-1} , T_4 - VC @ 2 t ac^{-1} + VCE, T_5 - VC @ 2 t ac^{-1} + VW and T_6 - VC @ 2 t ac^{-1} + VCE + VW. About 40 days old seedlings of *A. paniculata* were transplanted in the cement pots. After cultivation practices were carefully carried out up to 90 days. The different growth parameters *viz.*, shoot length (cm), root length (cm), number of branches ($plant^{-1}$), shoot biomass (g $plant^{-1}$) and root biomass (g $plant^{-1}$) were recorded on 45 and 90 DAT.

Antimicrobial activity: The different plant parts of *A. paniculata viz.*, leaf, stem and root were separately collected from all the treatments followed by shade drying for two weeks and then ground into powder. The powder materials were packed in a separate plastic zip covers and stored under refrigeration until use. About 5 gram of each powder sample was extracted with 25 ml of methanol and kept in shaker for overnight, the extract were concentrated using rotary evaporator at 45º C and stored at 4º C in air tight container until use. Methanolic extracts of *A. paniculata* were screened for antimicrobial activity using disc diffusion assay method according to [15].

The log phase cultures of bacteria (*Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Bacillus cereus*) were spread uniformly on the separate Petri plates containing Muller Hinton agar. Discs with methanolic extracts of leaf, stem and root were placed on the agar surface with sterile forceps and gently pressed down to ensure complete contact of the disc with agar surface. Positive control tetracycline discs were also placed in all the plates. The plates were incubated at 37°C for 24 h and triplicates were maintained for all treatments respectively. After incubation period, the zones of inhibition around the discs were measured in mm using Hi Antibiotic zone scale. About 48 h old cultures of fungi (*Aspergillus niger*, *Aspergillus flavus* and *Candida albicans*) were spread on the surface of Sabouraud's dextrose agar. Discs with methanolic extracts of leaf, stem and root were placed on the agar surface as described above with antifungal agent (fluconazole) as a positive control. Plates were incubated at 28°C for 72 h. After incubation period, the zones of incubation in diameter (mm) were measured.

Statistical Analysis: All the reported data are the arithmetic means of three replicates. Statistical analysis of plant growth characters was carried out through analysis of variance (ANOVA) according to [16]. Antimicrobial activity data were expressed as mean ± standard deviation (SD), where n = 3.

RESULTS AND DISCUSSION

In the present study, vermicompost obtained from cow dung was used for preparation of vermicompost extract and vermiwash, as cow dung has been considered as most preferred feed material [11, 12, 17, 18] for earthworms. The effect of application of vermicompost (VC), vermicompost extract (VCE) and vermiwash (VW) at different combinations on certain growth parameters such as shoot length, root length, number of branches, shoots dry weight, root dry weight and total plant dry weight on 45 and 90 DAT were recorded (table 1). The treatment T_6 (VC + VCE + VW) was performed superior over other treatments regarding all parameters tested and it was followed by T_4 (VC + VCE), T_5 (VC + VW), T_3 (VC), T_2 (RDF) and T_1 (control). The treatment T_6 which received the combined application of vermicompost and its related products (vermicompost extract and vermiwash) recorded maximum shoot length (63.56 cm), root length (23.49 cm), number of branches (20.57 plant^{-1}), shoots dry weight (20.36 g plant^{-1}), root dry weight (8.18 g plant^{-1}) and total plant dry weight (28.54 g plant^{-1}) and was found to be significantly higher than the treatment T_4 on 90 DAT with values as 61.25 cm, 22.04 cm, 19.19 plant^{-1}, 18.79 g plant^{-1}, 7.55 g plant^{-1} and 26.34 g plant^{-1} respectively.

It was evidenced that the application of VC @ 2 t ac^{-1} (T_3) significantly improves all the growth parameters than the recommended dose of inorganic fertilizer (T_2). When the vermicompost application was supplemented with VCE and VW, significant improvement in growth and development of *A. paniculata* was observed. The treatment T_6 (VC + VCE + VW) recorded about 15.12% increase over T_2 (RDF) on shoot length on 90 DAT. The treatment was followed by T_4 (10.94%), T_5 (8.34%) and T_3 (4.15%). It was found that the treatments T_4 and T_5 are statistically on par with each other. Similar levels of observations were recorded in all other growth parameters among the treatments.

Table - 1: Effect of vermicompost, vermicompost extract and vermiwash on growth and yield characters of *A. paniculata*

Treatments	Shoot length (cm)		Root length (cm)		Number of branches (plant^{-1})		Shoot dry weight (g plant^{-1})		Root dry weight (g plant^{-1})		plant dry weight (g plant^{-1})	
	45 DAT	90 DAT	45 DAT	90 DAT	45 DAT	90 DAT	45 DAT	90 DAT	45 DAT	90 DAT	45 DAT	90 DAT
T_1	18.02	27.37	6.75	9.40	4.35	7.92	3.86	7.59	1.36	2.95	5.22	10.54
T_2	33.45	55.21	11.67	18.24	9.43	15.65	9.12	14.67	3.88	5.90	13.00	20.57
T_3	35.25	57.50	12.72	19.71	10.48	16.97	10.30	16.24	4.34	6.52	14.64	22.76
T_4	38.14	61.25	14.24	22.04	12.31	19.19	12.06	18.79	5.07	7.55	17.13	26.34
T_5	37.01	59.82	13.71	21.17	11.55	18.36	11.44	17.82	4.79	7.13	16.23	24.95
T_6	39.89	63.56	15.26	23.49	13.37	20.57	13.21	20.36	5.50	8.18	18.71	28.54
S.E.	0.85	1.12	0.47	0.69	0.50	0.67	0.55	0.75	0.20	0.29	-	-
C.D.(P=0.05)	1.71	2.27	0.96	1.40	1.03	1.35	1.11	1.53	0.41	0.59	-	-

Application of vermicompost obtained from coir pith can be used for the improvement of *A. paniculata* under saline soils [19]. The application of vermicompost as individual and combination with other biofertilizers significantly improves the seed quality attributes and andrographolide content of *A. paniculata* [20, 21]. It may be attributed that application of highly humified vermicompost may enhance the growth and yield parameters of many crops since it contains macro and micro nutrients in the available form, beneficial microorganisms, growth promoting substances, tolerance to various abiotic stresses and suppression of plant pathogens.

The antimicrobial (antibacterial and antifungal) activities of methanolic extract of different parts (leaf, stem and root) of *A. paniculata* were studied by disc diffusion assay against certain important human

pathogens *viz., E. coli, S. typhi, S. aureus* and *B. cereus* (bacteria) and *A. niger, A. flavus* and *C. albicans* (fungi). Their antimicrobial activities were compared with standard antibacterial (tetracycline) and antifungal (fluconazole) antibiotics (figures 1 to 6).

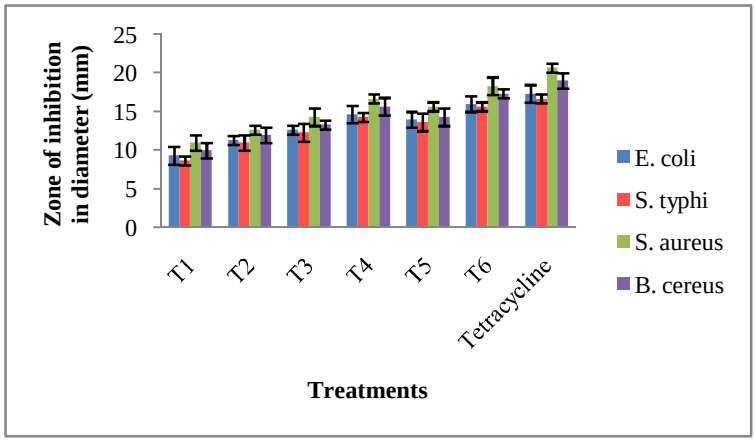

Fig. 1: Antibacterial activity of leaf extract of *A. paniculata*

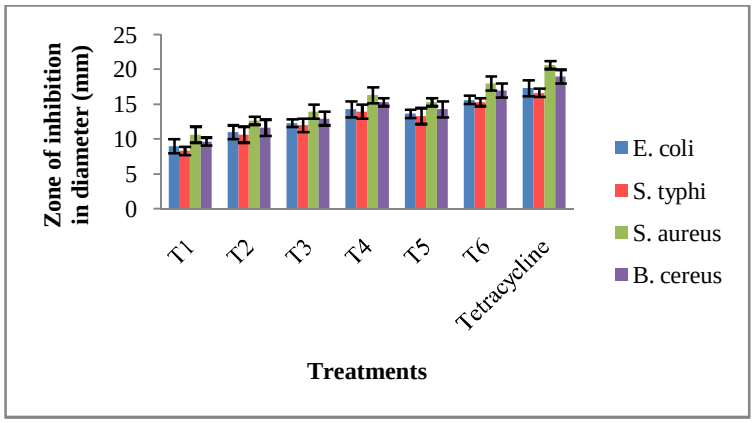

Fig. 2: Antibacterial activity of stem extract of *A. paniculata*

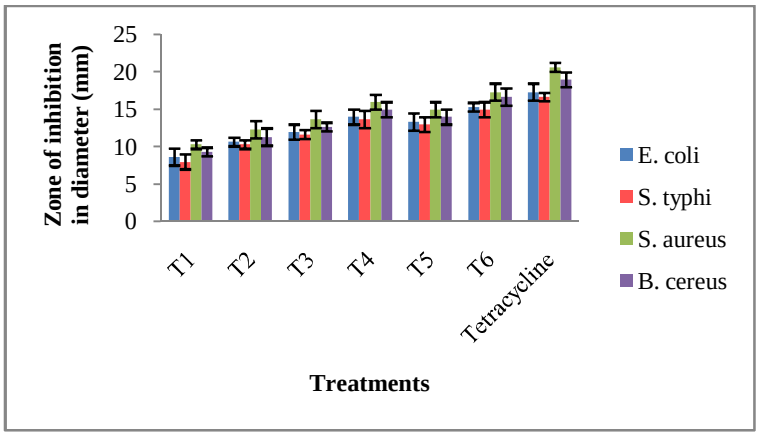

Fig. 3: Antibacterial activity of root extract of *A. paniculata*

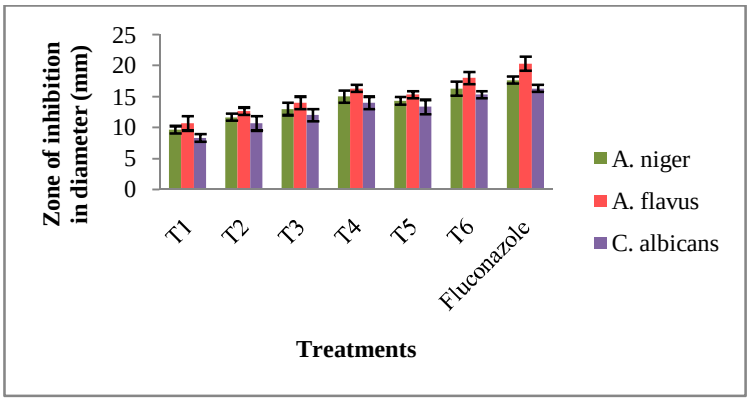

Fig. 4: Antifungal activity of leaf extract of *A. paniculata*

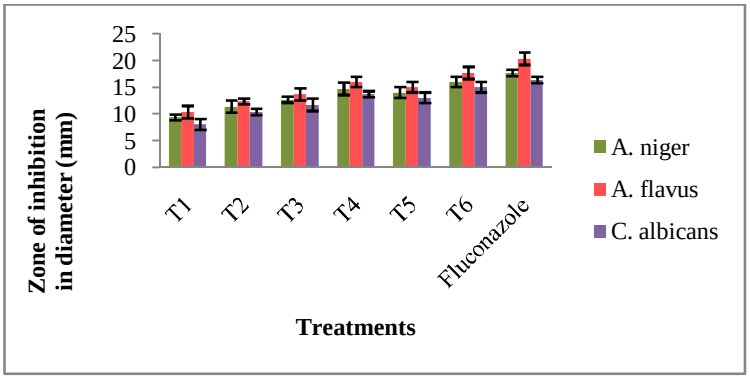

Fig. 5: Antifungal activity of stem extract of *A. paniculata*

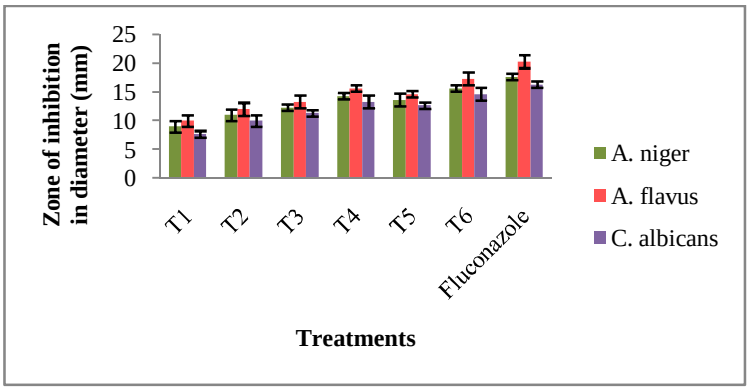

Fig. 6: Antifungal activity of root extract of *A. paniculata*

The present study clearly revealed that the methanolic extract of leaves showed higher inhibitory action on the pathogens tested than that of stem and root extract of *A. paniculata* in all the treatments. It was observed that, the antibacterial antibiotic (tetracycline) showed higher zone of inhibition against all four bacterial pathogens tested *viz.*, 17.33±1.15 mm (*E. coli*), 16.67±0.58 mm (*S. typhi*), 20.67±0.58 mm (*S. aureus*) and 19.00±1.00 mm (*B. cereus*). Among the different treatments employed, the T_1 (control) performed poor antibacterial activity against all the pathogens. The treatment was followed by T_2 (RDF), T_3 (VC), T_5 (VW), T_4 (VCE) and T_6 (VC + VCE + VW). Among the treatments, the maximum inhibition zone of leaf extract of *A. paniculata* was recorded as 18.33±1.15 mm against *S. aureus* and followed by 17.33±0.58 mm (*B. cereus*), 16.00±1.00 mm (*E. coli*) and 15.67±0.58 mm (*S. typhi*) were recorded.

Similar to antibacterial activity, the standard fluconazole showed maximum of 20.33±1.15 mm (*A. flavus*), 17.67±0.58 mm (*A. niger*) and 16.33±0.58 mm (*C. albicans*). The methanolic extract of leaf obtained from the treatment T_6 showed higher antifungal activity within the treatments.

In all the treatments, it was observed that methanolic extract of different parts of *A. paniculata* treated with RDF (T_2) performed poor than VC (T_3). Furthermore, it was observed when vermicompost was supplemented with novel organic products like VCE and VW, their inhibitory action is slightly improved.

The antimicrobial activity of leaf and stem extract of *A. paniculata* was higher than root extract. It may be due to the presence of active principle compounds like diterpene lactones (andrographolide, neoandrographolide, deoxyandrographolide, 14-deoxy-11, 12-didehydroandrographolide and andrograpanin), alkaloids, flavonoids, glycosides, steroids, phenols, tannins and saponins.

APPLICATIONS

Application of vermicompost, vermicompost extract and vermiwash significantly improves the growth and yield characters of *A. paniculata*. They also increase the antibacterial and antifungal activity of methanolic extract of leaf, stem and root of *A. paniculata.*

CONCLUSION

The present study concluded that application of VC significantly promotes the growth and antimicrobial activity of *A. paniculata* than recommended dose of inorganic fertilizer. When VC was supplemented with VCE and VW further enhancement in the growth parameters and antimicrobial activity were observed. Hence, application of VC, VCE and VW can be recommended in *A. paniculata* cultivation to enhance the quality and quantity attributes.

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