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Antibacterial Activity of Microbial Treated *Andrographis paniculata* (Burm.f.) Wall. Nees under Drought Stress

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ABSTRACT

Andrographis paniculata plants were treated with various plant growth promoting microorganisms incorporated into the experimental field included *Bacillus megaterium* (B), *Trichoderma harzianum* (T), *Funneliformis mosseae* (F) and consortia of *F. mosseae* and *T. harzianum* (FT), *B. megaterium* and *T. harzianum* (BT), *F. mosseae* and *B. megaterium* (FB) exposed to irrigated and drought soil conditions were tested for their antimicrobial activities on gram positive and gram negative bacteria. The methanolic extract prepared after different microbial treatments of *A. paniculata* plants under drought stress was more potent in inhibiting the bacterial growth of both gram positive (*Paenibacillus polymyxa*) and gram negative bacterium (*Xanthomonas campestris*) than under irrigated condition. Experimental results showed compatibility between consortia of *F. mosseae* and *B. megaterium* and was found to enhance antimicrobial activity of *A. paniculata* plant maximally among all other microbial treatments under drought stress.

Keywords : Antimicrobial Activity, *Andrographis Paniculata,* Drought, Gram Positive Bacteria, Gram Negative Bacteria, Microbial Treaments

I. INTRODUCTION

Andrographis paniculata (Burm.f.) Wall. Nees of Acanthaceae family is an annual herb widely distributed throughout Indian plains and South-East Asia. It contains principle active compounds like andrographolide and neoandrographolide [1]. This plant possess numerous properties like anticancerous activity [2], antidiabetic [3], antihepatoxic [4], antioxidant activity [5], cardiovascular activity [6], wound healing, vasorelaxant property [7], neurogenerative activity [8], hypoglycemic activity [9], antifungal [10], antiviral [11] and antibacterial activity [12].

Bacterial resistance development to presently available antibiotics has made it necessary to look for new antimicrobial agents. Herbal extracts is frequently used to improve health as well as to prevent microbial infections [13]. Ubiquity of multiple drug resistance has led to new areas of research on natural phytochemicals obtained from plants in form of herbal extract, are being investigated as a source of antimicrobial agents.

Literature reveals that plant phytochemicals are strongly affected by abiotic stress [14]. Drought is among the major abiotic factor which will affect plant cultivation. Plant growth promoting microbes are employed as sustainable methods to deal with the drought stress in the plants which alter the physiological activity of plant [15]. Studies on effect of drought under microbial treatments on antibacterial activity of *A. paniculata* are lagging. These factors are hypothesized to alter the phytochemical constituents of plants which in turn may affect their antibacterial activity which is aimed in present study. The present study was undertaken to find out the antibacterial potentiality of the methnolic extract of the whole plant part (root, stem and leaves) against Gram positive and Gram negative bacteria.

II. MATERIAL AND METHODS

Plant material and growth condition

A. paniculata plant with voucher specimen no. – 91924 of was deposited at Botanical Survey of India, Allahabad. The whole plant part (root, stem, leaves, flower, seed) was oven dried for 72 h at 40 ºC and powdered in mechanical grinder. The plant was grown in field with 6 different microbial treatments as mentioned 1. *Bacillus megaterium* (B), 2. *Trichoderma harzianum* (T), 3. *Funneliformis mosseae* (F) and consortia of 4. *Funelliformis mosseae* and *Trichoderma harzianum* (FT), 5. *Bacillus megaterium* and *Trichoderma harzianum* (BT), 6. *Funelliformis mosseae* and *Bacillus megaterium* (FB) 7. Control without any innoculation exposed to irrigated (daily watered) and drought (watering after 3 days) soil conditions.

Plant extract preparation

Finely powdered 20 g of plant material was subjected to extraction in 250 ml methanol for 48 h in soxhlet apparatus. The methanol extract of different microbial treated was collected, filtered and concentrated in vaccum under rota-evaporator and kept in dessicator.

Antibacterial assay

The methanolic plant extract was tested for the antimicrobial activity against two bacterial strains (*Paenibacillus polymyxa* and *Xanthomonas campestris*) by disc diffusion method. The strain was collected from IAS, B.H.U. and IARI, New Delhi respectively. The pure culture of these bacterial strains were sub-cultured as slant nutrient agar culture and stored at 4 ºC. The bacterial cultures were inoculated into the nutrient broth media and incubated at 35 ± 2 ºC for 24 h. The turbidity of resulting suspensions was diluted with nutrient broth media to obtain a transmittance of 25 % at 580 nm. The level of turbidity is adjusted to 1.5×10^8 cfu/ml comparable to 0.5 McFarland turbidity standard. Later, 10 µl of these test microorganisms were swabbed into solidified nutrient agar media from the 24 h old bacterial culture by spread plate method. The filter paper discs (5 mm in diameter) were infused with the plant extracts and placed on test microorganisms seeded plates. Streptomycin sulphate (10 µg/ml) was used as positive control and methanol solvent was used (100 µg/ml) as negative control. The antibacterial assay plates were incubated at 37 ºC for 24 h and diameter of inhibition zones were measured.

Statistical analysis

For each experiment three replicates were used and repeated for three times independently. The mean values were represented as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) and separation between means were calculated by using Tukey's Test at $p < 0.05$ (SPSS software, version 16) under irrigated and water stress conditions.

III. RESULTS

The antibacterial activity of the various microbial treated plant extract prepared in methanolic solvent exhibited different levels of antibacterial activity against *Paenibacillus polymyxa* and *Xanthomonas campestris.* The maximum zone of inhibition was reported in dual consortia (FB) *X. campestris* (9.25 ± 0.5 mm) under irrigated soil condition (Table 1) while same plant consortia (FB) grown under drought soil condition showed significant highest inhibition zone of (11.5 ± 0.50 mm) by *P. polymyxa* and (10.25± 0.5 mm) by *X. Campestris* (Table 2). Maximum antibacterial activity by methanolic extract of *A. paniculata* exposed to FB treatment under drought soil condition showed 36.36 % and 41.37 % of inhibition in *P. polymyxa* and *X. campestris* respectively while same treatment under irrigated soil condition showed 23.52 % and 48 % of inhibition in *P. polymyxa* and *X. campestris* respectively. The andrographolide (AND) showed inhibition zone of (7.45 ± 0.52 mm) in *P. polymyxa* and (7.12 ± 0.25 mm) in *X. campestris* under irrigated condition while same standard showed inhibition of (8.75 ± 0.50 mm) in *P. polymyxa* and (8.25± 0.50 mm) in *X. Campestris* under drought condition. The standard antibiotic streptomycin showed inhibition zone of (8.25± 0.50 mm) in *P. polymyxa* and *X. campestris* (7.97 ± 0.12 mm) under irrigated condition while the same showed zone inhibition of (11.5 ± 0.50 mm) by *P. polymyxa* and *X. campestris* (10.25 ± 0.50 mm) under drought condition.

Table 1: THE ANTIBACTERIAL ACTIVITY SHOWN BY METHANOLIC EXTRACT OF *A. PANICULATA* EXPOSED TO DIFFERENT MICROBIAL TREATMENTS UNDER IRRIGATED SOIL CONDITION

Zone of inhibition (mm)		
Treatments	*Paenibacillus polymyxa* (Gram positive bacteria)	*Xanthomonas campestris* (Gram negative bacteria)
Bacillus megaterium (B)	9.0 ± 0.81 c	7.5 ± 0.4 bc
Trichoderma harzianum (T)	8.8 ± 0.50 c	7.2 ± 0.5 b
Funelliformis mosseae (F)	7.5 ± 0.58 ab	7.8 ± 0.5 bc
Funelliformis mosseae *Trichoderma harzianum* (FT)	8.0 ± 0.81 abc	8.8 ± 0.5 d
Bacillus megaterium *Trichoderma harzianum* (BT)	9.0 ± 0.81 c	7.7 ± 0.4 bc
Funelliformis mosseae *Bacillus megaterium* (FB)	10.50 ± 0.58 d	9.25 ± 0.5 d
Control (C)	8.5 ± 0.58 bc	6.25 ± 0.5 a
Andrographolide (AND)	7.45 ± 0.52 ab	7.12 ± 0.25 b
Methanol	7.0± 0.81 a	6.32 ± 0.394 a
Streptomycin	8.25 ± 0.50 bc	7.97 ± 0.12 c

Mean ± standard deviation (n = 3). Values in columns sharing the same letter do not differ significantly ($p < 0.05$) as determined by the Tukey's test by one way ANOVA.

Table 2: THE ANTIBACTERIAL ACTIVITY SHOWN BY METHANOLIC EXTRACT OF *A. PANICULATA* EXPOSED TO DIFFERENT MICROBIAL TREATMENTS UNDER DROUGHT SOIL CONDITION

Zone of inhibition (mm)		
Treatments	*Paenibacillus polymyxa* (Gram positive bacteria)	*Xanthomonas campestris* (Gram negative bacteria)
Bacillus megaterium (B)	11.25 ± 0.57 cd	7.75 ± 0.50 abc
Trichoderma harzianum (T)	10.5± 0.57 c	8.25 ± 0.50 bcd
Funelliformis mosseae (F)	9.25 ± 0.50 b	8.75 ± 0.50 d
Funelliformis mosseae Trichoderma harzianum (FT)	8.5 ± 0.58 ab	9.75 ± 0.50 e
Bacillus megaterium Trichoderma harzianum (BT)	10.5 ± 0.58 c	8.5 ± 0.58 cd
Funelliformis mosseae Bacillus megaterium (FB)	11.5 ± 0.50 d	10.25 ± 0.50 e
Control (C)	8.25 ± 0.50 a	7.25 ± 0.50 a
Andrographolide (AND)	8.75 ± 0.50 ab	8.25 ± 0.50 bcd
Methanol	8.5 ± 0.58 ab	7.5 ± 0.58 ab
Streptomycin	9.25 ± 0.50 b	8.25 ± 0.50 bcd

Mean ± standard deviation (n = 3). Values in columns sharing the same letter do not differ significantly ($p < 0.05$) as determined by the Tukey's test by one way ANOVA.

IV. DISCUSSION

The current study was based on the hypothesis that PGPRs induces metabolic adjustment in plants leading to augmented antimicrobial activity. The antimicrobial properties of the kalmegh plant may be due to presence of active metabolite content like carbohydrate, tannins, flavonoids, saponins and andrographolide of the plant. It showed antibacterial activity against *P. polymyxa* and *X. Campestris* in plants under the drought stress. Beneficial effects of PGPM are mostly enhanced when they are co-inoculated and this depends on the synergistic effect of the fungus-bacterium [15]. The methanolic extract might be properly dissolve the plant constituents like andrographolide, neoandrographolide etc along with other phytochemicals as a whole thus showed more activity in comparison of purified andrographolide. The andrographolide showed antibacterial against *P. polymyxa* and *X.campestris* but the whole plant extract of *A. paniculata* showed relatively more antibacterial activity due to presence of many phytochemicals such as terpenoids, lactones and flavonoids. The methanolic extract of *A. paniculata* displayed antimicrobial activity against the bacterial strain. Mishra et al., [16] reported that ethanolic extract of *A. paniculata* inhibited gram positive and negative bacterial growth. Malahubban et al., [17] stated maximum inhibitory effects against *Bacillus cereus* and *Staphylococcus aureus* in methanolic leaf extract. In this study, the gram positive bacteria showed more pronounced result than gram negative bacteria under drought condition in *P. polymyxa.* It might be due to differences in the morphological constitution of *P. polymyxa* and *X. campestris. P. polymyxa* being gram positive possessing only outer peptidoglycan layer are more susceptible and acts an effective permeability barrier [18] while gram negative *X.*

campestris have an outer phospholipid membrane with a lipopolysaccharide component which makes plant extracts impermeable to cell wall [19]. This study reports that the gram positive bacteria after giving dual microbial treatment of FB in *A. paniculata* under drought stress performed better than same treatment in irrigated condition.

V. CONCLUSION

The present study shows an increased antimicrobial response of *A. paniculata* medicinal plant to drought stress under microbial inoculation of *F. mosseae* and *B. megaterium*. The consortia elicited the antibacterial activity as well as phytochemical compounds in plant thereby providing it with better capabilities to use *A. paniculata* plant as an antimicrobial agent under drought stress.

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VII. REFERENCES

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