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Antimicrobial activity of *Stevia rebaudiana Bertoni* leaves conferring disease resistance to wheat (*Triticum aestivum* L.)

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Abstract
An investigation was carried out at Banaras Hindu University, Varanasi to study the effect of *Stevia rebaudiana* on physiological and biochemical attributes of wheat conferring disease resistance. In the present investigations even medicinal plants were taken viz., *Rauwolfia serpentina, Gymnema sylvestre, Terminalia arjuna, Stevia rebaudiana, Strychnosnux-vomica, Tinospora cordifolia* and *Psoralea corylifolia* and treated against one bacterial sp. (*Xanthomonas* spp.), and four fungal pathogen (*Bipolaris* spp., *Curvularia* spp., *Fusarium* spp. and *Rhizoctoniasolani.*).*Stevia rebaudiana* found best among these medicinal plants. In the present investigation different biochemical parameters were carried out i.e. APX, TPC, PAL, MDA, H_2O_2 and SOD to observe and relate antimicrobial activity of wheat and found that APX activity decreased in wheat. The TPC activity was significantly increased after spray of stevia leaf extract which indicate that crops showed better resistance against the pathogen while PAL activity decreased. On the other hand MDA and SOD activity was also significantly increased.

Keywords: *Stevia rebaudiana*, pathogen, antimicrobial activity, disease resistance

Introduction
From ancient times many medicinal plants represent an excellent source of antimicrobial agents and the world health organization has estimated about 80% of the total world population to be relying on herbal medications. The potential of plants to have therapeutic properties can be imagined from the fact that today almost 25% of pharmaceutical prescriptions in United States have one of the constituents drawn from plants. Some important medicinal plants viz. *Rauwolfia serpentina*, *Gymnema sylvestre*, *Terminalia arjuna*, *Stevia rebaudiana*, *Strychnosnux-vomica*, *Tinospora cordifolia* and *Psoralea corylifolia* well known for their medicinal properties. These plants are used as antimicrobial agents since time immemorial. One of the reasons of increasing inclination towards herbal medications is upsurge in the antibiotic resistance which is one of the menacing issues of the medical world nowadays. Prioritizing herbal medicine is attributed to its minimal side effects and stupendous potential against treatment of infectious diseases in humans but have minor role in plant diseases. Keeping this in view, present investigation has been taken to study the antimicrobial effect of *Stevia rebaudiana* on fungal and bacterial diseases of wheat.

Material and Method
Plant materials: *Rauwolfia serpentina, Gymnema sylvestre, Terminalia arjuna, Stevia rebaudiana, Strychnosnux-vomica, Tinospora cordifolia* and *Psoralea corylifolia* were obtained from, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi.

Test organisms: *Xanthomonas spp.* was used to test antibacterial activity, *Bipolaris spp., Curvularia spp., Fusarium spp. and Rhizoctonia solani* were used for antifungal activity were collected from Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, BHU, Varanasi.

Preparation of Stevia plant extract
The *Stevia rebaudiana* leaves were dried in shade and grined into powder was used for extraction. 25 g of air-dried powder of leaves was immersed in 100 mL of organic solvents such as ethyl acetate, acetone, chloroform and distilled water in conical flask. It was incubated at room temperature for 48 hours at 150 rpm on an orbital shaker. The suspension was filtered and concentrated to dryness at 40^0C in hot air oven and the residue was dissolved in 0.1 ml of ethanolic solvent was used or antimicrobial activities.

Screening of antifungal activity

Media used: Potato dextrose agar (Hi Media) Preparation of inoculum: Potato dextrose agar (HiMedia) was plated. After solidification about 0.5 ml of culture suspension was spread on the agar medium. All the plates were incubated at 25^0C for 4 days. The experiments were performed in triplicates. The growth of the fungal cultures was measured and compared with the respective control plates.

Screening of antibacterial activity

Media used: Nutrient agar (Hi Media)

Preparation of inoculum: Stock cultures were maintained at 4^0C on nutrient agar (Hi Media) slants. Active cultures for experiments were prepared by transferring a loopful of culture to nutrient Agar slant (Hi Media), incubated at 37^0C hours for bacterial proliferation.

Agar-well diffusion method

Agar well bioassay was employed for testing antibacterial activity of *Stevia rebaudiana* leaves (Linday, 1962). Each extracts were made to a final concentration of 50 mg/mL. 24 hour old cultures of test organisms (0.05 mL) were seeded onto Mueller Hinton agar (HiMedia) plate and uniformly spread with a spreader. Wells (5mm) were made in the agar plate with a sterilecork borer. The plant extract was introduced into the well and the plates were incubated at 37^0C for 24 hours. The antibacterial activity of the plant extract was determined by measuring the diameter of the inhibition zone. Controls contained only Di Methyl Sulfoxide (DMSO). The antibacterial assay for each of the extracts against all microorganisms tested was performed in triplicates.

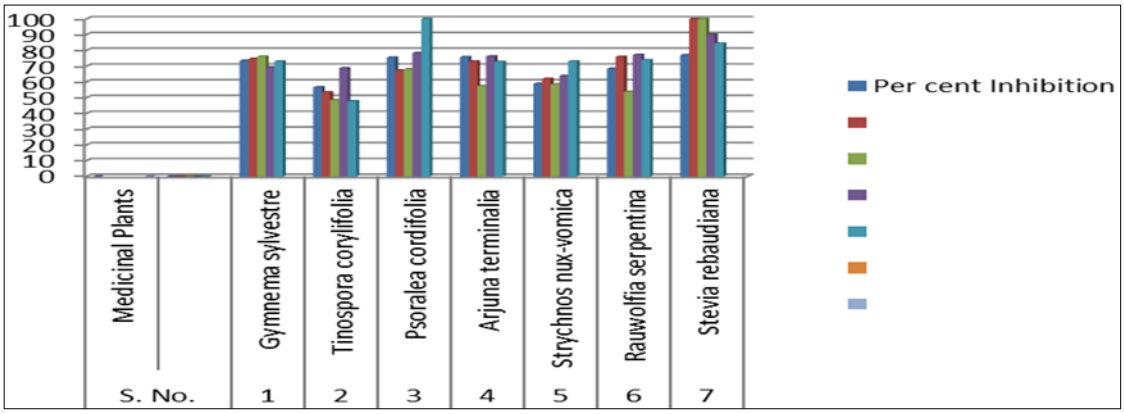
After screening of all the medicinal plants, the pathogen's percent growth inhibition were recorded and selected the best performing medicinal plants for antimicrobial activity which have highest per cent inhibition, among which *stevia rebaudiana* shows best results. Ascorbate peroxidase (APX), Super Oxide Dismutase (SOD) activity, Malondialdehyde (MDA) content, Total Phenol Content (TPC), Phenyl alanine ammonia Lyase (PAL) were estimated using standard protocol.

Result and Discussion

In the present investigation seven medicinal plants were taken viz., *Rauwolfia serpentina*, *Gymnema sylvestre*, *Terminalia arjuna*, *Stevia rebaudiana*, *Strychnosnux-vomica*, *Tinospora cordifolia* and *Psoralea corylifolia* and treated against one bacterial sp. (*Xanthomonas spp*.), and four fungal pathogen (*Bipolaris spp*., *Curvularia spp*., *Fusarium spp*. and *Rhizoctonia solani*.). After screening of all the medicinal plants, the pathogen's percent growth inhibition was recorded and selected the best performing medicinal plants for antimicrobial activity which have highest per cent inhibition.

Table 1: Effect of different medicinal plants leaf extract percent (%) on growth inhibition of the pathogen

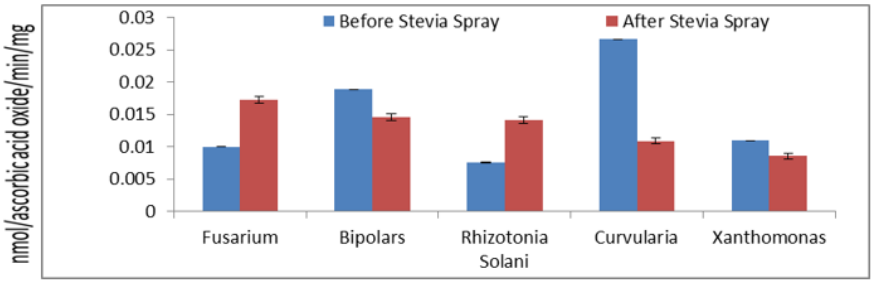
S. No.	Medicinal Plants	Per cent Inhibition				
		Fungal Pathogen				Bacterial Pathogen
		***Fusarium* spp.**	***Bipolaris* spp.**	***Rhizoctonia solani***	***Curvularia* spp.**	***Xanthomonas* spp.**
1	*Gymnema sylvestre*	73.46	74.68	76.06	69.32	73.00
2	*Tinospora corylifolia*	56.65	53.41	48.49	68.79	47.71
3	*Psoralea cordifolia*	75.42	67.29	68.08	78.41	100
4	*Arjuna terminalia*	75.85	72.99	57.45	76.25	72.79
5	*Strychnosnux-vomica*	58.88	62.03	58.50	63.84	73.05
6	*Rauwolfia serpentina*	68.42	75.97	53.67	77.12	73.87
7	*Stevia rebaudiana*	76.95	100	100	90.05	84.40



From the (Table 1), *Stevia rebaudiana* was found better in respect to high per cent growth inhibition for the antimicrobial activity than all the rest medicinal plants. Thus, *Stevia rebaudiana* was taken for the antimicrobial activity to carry out research in wheat.

Activity of Ascorbate Peroxidase (APX)

When the fungal pathogens were infected to see the incidence of disease, after few days the extract of *stevia* was sprayed to observe the antimicrobial activity in wheat. The activity of APX was decreased up to 29% in *Bipolaris* spp., 42% increase in *Fusarium* spp., 47% increase in *Rhizoctonia solani* and 144% decrease in *Curvularia* spp. in wheat. While in bacterial pathogen infected by *Xanthomonas* spp., the activity of APX was found to decrease with 28% in wheat. One of the earliest cellular responses following successful pathogen recognition is oxidative burst involving production of ROS. Recognition of a variety of pathogens leads to generation of O_2•−, or its dismutation product H_2O_2 inapoplast (Grant *et al*., 2000) [4].



Pathogen Infection

Total Phenol Content (TPC) Activity

When the fungal pathogens were infected to see the incidence of disease, after few days the extract of *stevia* was sprayed to observe the antimicrobial activity in wheat. The activity TPC was increased up to 78% in *Bipolaris* spp., 66% increase in *Fusarium* spp., 91% increase in *Rhizoctonia solani* and 235% decrease in *Curvularia* spp. while in bacterial pathogen infected by *Xanthomonas* spp., the activity of TPC was found to decrease with 21% in wheat. Polyphenol substances that are widely present in plants are known to play an important role for antioxidant effects and defence action in the plant, and phenolic compounds generally have different physico-chemical properties and physiological functions depending on their structure (Gupta and Tandon, 2004) [5]; (Jayaraman, 2011) [6].

Table 3: Total Phenol Content (TPC) in wheat

S. No.	Pathogen inoculated in wheat	Before spray (stevia extract)	After spray (stevia extract)
1	*Fusarium* spp.	1.131±0.023	3.315±0.037
2	*Bipolaris* spp.	1.385±0.034	0.414±0.039
3	*Rhizotonia Solani*	0.090±0.015	0.410±0.031
4	*Curvularia* spp.	0.043±0.019	0.485±0.114
5	*Xanthomonas* spp.	0.235±0.023	0.191±0.014

Activity of Phenylalanine Ammonia Lyase (PAL)

When the fungal pathogens were infected to see the incidence of disease, after few days the extract of *stevia* was sprayed to observe the antimicrobial activity in wheat. The activity of PAL was decreased up to 27% in *Bipolaris* spp., 29% decrease in *Fusarium* spp., 36% decrease in *Rhizoctonia solani* and 51% increase in Curvularia spp. in wheat while in bacterial pathogen infected by *Xanthomonas* spp., the activity of PAL was found to decrease by 25 per cent. These experimental findings indicated that increase in PAL activity shows m Above findings were supported by (Kagale *et al*., 2004) [7] who reported higher activity of PAL, peroxidase, chitinase, β-1,3-glucanase and increase in level of phenols in rice leaves treated with Datura leaf extract and inoculated with *Rhizoctonia solani* or *Xanthomona soryzae* pv. *Oryzae* resistance against pathogens.

Table 4: Activity of Phenylalanine Ammonia Lyase (PAL) in wheat

S. No.	Pathogen inoculated in wheat	Before spray (stevia extract)	After spray (stevia extract)
1	*Fusarium* spp.	5.656±0.031	4.371±0.026
2	*Bipolaris* spp.	4.799±0.023	3.784±0.005
3	*Rhizotonia Solani*	4.791±0.038	3.523±0.008
4	*Curvularia* spp.	2.457±0.037	4.989±0.121
5	*Xanthomonas* spp.	5.982±0.040	4.801±0.026

Activity of Malondialdehyde (MDA)

When the fungal pathogens were infected to see the incidence of disease, after few days the extract of *stevia* was sprayed to observe the antimicrobial activity in wheat. The activity of MDA was increased up to 80% in *Bipolaris* spp., 72% increase in *Fusarium* spp., 3% increase in *Rhizoctonia solani* and 13% increase in *Curvularia* spp., while in bacterial pathogen infected by *Xanthomonas* spp., the activity of MDA was found to increase by 20% in wheat. Above data represent that MDA content was significantly increased. It enhanced plant membrane per-oxidation as reported by many investigators (Bogatek & Gniazdowska, 2007).

Table 5: Activity of Malondialdehyde (MDA) in wheat

S. No.	Pathogen inoculated in wheat	Before spray (stevia extract)	After spray (stevia extract)
1	*Fusarium* spp.	0.049±0.024	0.174±0.020
2	*Bipolaris* spp.	0.090±0.021	0.452±0.011
3	*Rhizotonia Solani*	0.060±0.016	0.062±0.023
4	*Curvularia* spp.	0.062±0.031	0.071±0.019
5	*Xanthomonas* spp.	0.052±0.026	0.065±0.024

Activity of Hydrogen Peroxide (H_2O_2)

When the fungal pathogens were infected to see the incidence of disease, after few days the extract of *stevia* was sprayed to observe the antimicrobial activity in wheat. The activity of $H_2O_2$was increased up to 16% in Bipolaris spp., decreased 3% in *Fusarium* spp., 19% decrease in *Rhizoctonia solani* and 95% increase in *Curvularia* spp. while in bacterial pathogens infected by *Xanthomonas* spp., the activity of H_2O_2 was found to be decreased with 51 per cent. One of the earliest cellular responses following successful pathogen recognition is oxidative burst involving production of ROS. Recognition of a variety of pathogens leads to generation of O_2•−, or its dismutation product H_2O_2 in apoplast (Grant *et al*, 2000) [4]. It has been observed by other researcher that higher H_2O_2concentrations in *Viciafaba* leaves infected with bean yellow mosaic virus than those of the corresponding controls (Kumar *et al*., 2008) [8].

Table 6: Activity of Hydrogen Peroxide (H_2O_2) in wheat

S. No.	Pathogen inoculated in wheat	Before spray (stevia extract)	After spray (stevia extract)
1	*Fusarium* spp.	55.28±0.763	53.73±0.293
2	*Bipolaris* spp.	42.64±0.288	51.04±1.547
3	*Rhizotonia Solani*	57.76±3.087	48.64±1.257
4	*Curvularia* spp.	1.36±1.310	28.05±1.00
5	*Xanthomonas* spp.	64.56±1.386	42.74±0.716

Activity of Superoxide Dismutase (SOD)

When the fungal pathogens were infected to see the incidence of disease, after few days the extract of stevia was sprayed to observe the antimicrobial activity in wheat. The activity of SOD was decreased up to 78% in *Bipolaris* spp., 91% decrease in *Fusarium* spp., 12% decrease in *Rhizoctonia solani* and 14% increase in *Curvularia* spp. while in bacterial pathogens infected by *Xanthomonas* spp., the activity of SOD was found to be decreased by 63 per cent. Above data showed that the activity of SOD was increased after pathogen $H_2O_2$and O_2, and is the first enzyme to deal with oxy-radicals (Piluzza *et al*., 2011) [9].

Table 7: Activity of Superoxide Dismutase (SOD) in wheat

S. No.	Pathogen inoculated in wheat	Before spray (stevia extract)	After spray (stevia extract)
1	*Fugarium* spp.	1.800±0.031	0.943±0.152
2	*Bipolaris* spp.	1.873±0.020	1.054±0.011
3	*Rhizotonia Solani*	1.654±0.04	1.474±0.050
4	*Curvularia* spp.	1.820±0.006	1.599±0.008
5	*Xanthomonas* spp.	1.750±0.021	1.076±0.008

Conclusion

In the present investigation different biochemical parameters were carried out i.e. APX, TPC, PAL, MDA, H_2O_2 and SOD to observe and relate antimicrobial activity of wheat and found that APX activity decreased in wheat. The TPC activity was significantly increased after spray of *stevia* leaf extract which indicate that crops showed better resistance against the pathogen while PAL activity decreased. On the other hand, MDA and SOD activity was also significantly increased. This study points to the probable antimicrobial potential of solvent extracts of *Stevia rebaudiana* leaves, reducing the incidence of pathogen infection in wheat plant.

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