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Prophylactic use of natural phage cocktail for plants and potential for human applications

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ABSTRACT

Background: Phages targeting specific bacteria have been used for control of bacterial pathogens in plants and humans. We report results of prophylactic use of a natural cocktail of phages on plants.

Materials and Methods: Water was collected from upper reaches of the Alaknanda river where more than 200 isolates of phages have been reported. The river water was collected in April 2020 before the onset of monsoons and stored with sediments. The water was sprayed twice weekly on spinach, cabbage and coriander plants. We did not isolate, identify or multiply the phages. We sprayed the water in its natural condition after diluting it to 10 percent with normal spring water that is used for irrigation in the area. Control plots were sprayed with normal spring water only.

Results: We find 47 percent higher yields in spinach, 6 percent in cabbage and 40 percent in coriander.

Conclusions: Natural phages of Ganga river could provide an inexpensive approach for the improvement of plant yields. Implications for prophylactic use of cocktail of phages for humans are discussed.

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1. Introduction

1.1. Use of phages in plants

The therapeutic and prophylactic efficacy of a phage cocktail was found to be effective in grapevines.¹ Phages were used as prophylactics by applying them to seeds before sowing.² A number of studies report the use of phages to treat specific plant pathogens.^{3–7} These studies indicate a potential for prophylactic use of phages in plants.

1.2. Cocktail phage therapy of plants

Koskella and Taylor report that broad-host-range phages are capable of suppressing target host populations in multispecies biofilms in plants.⁸ Meaden and Koskella report that treatment of bacteria with multiple phages was more effective. This emulates the common practice of

using wide-spectrum antibiotic treatments.⁴ We make the novel innovation of using naturally available phage cocktail for prophylactic treatment in plants without identifying the target bacteria, isolating the corresponding phages and making a targeted phage cocktail.

1.3. Natural phage cocktail

The National Environment Engineering Research Institute, Nagpur has reported that water of upper stretches of river Ganga contains more than 200 isolates of phages.⁹ The Institute of Microbial Technology, Chandigarh has reported that phages having specific bactericidal activity against clinical isolates and pathogens like Mycobacterium, Streptococcus, Pseudomonas, Yersinia, Salmonella, Staphylococcus, Klebsiella, Vibrio, Shigella, Clostridium, Acinetobacter, Erwinia, Aeromonas, Escherichia, Cronobacter, Enterobacteria, and Campylobacter etc. are found in the Ganga waters. Thus, here we have available

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here an inexpensive phage cocktail.

The use of phages to treat plant conditions is limited, among others, by the exposure to ultraviolet rays, dryness, and temperature fluctuations that are deleterious to the phages.³ These bottlenecks do not restrict the use of the natural phage cocktail since the phages in the river are stable in the natural conditions obtaining in the river water and sediments.

The aim of the research to investigate whether natural phage cocktails can be used for prophylactic use in plants was achieved successfully.

2. Materials and Methods

We collected the water and sediments from the Alaknanda river upstream of the confluence of Mandakini and Alaknanda rivers at Rudra Prayag. The site is located 160 kilometers from Haridwar where the river enters the plains. Alaknanda is the main tributary of the Ganga river. She is also known as Ganga river.

We collected water in April 2020 before the onset of monsoons and stored it with sediments. 1000 liters water was collected in virgin plastic containers and stored away from sunlight to prevent the development of algae. We postulated that the phages adsorbed in the sediments would clean-up the overlaying column of water of all coliforms.

We had the water tested at People's Science Institute, Dehradun in February 2021. One gram of fresh cow dung was diluted in 2 liters of distill water. 100 ml of the dilution was then added to one liter distil water. 100 ml of this "cow dung dosage" was introduced in 1 liter Ganga water. Then the sample was tested for the presence of fecal coliforms. The results are given in Table 1.

These results indicate that the phages in the Ganga water had cleaned-up the fecal coliforms. The figure of 440 MPN at 0 hours may be an outlier and we ignore it.

We did not identify or isolate the phages. We postulated that the large numbers of isolates of phages present in the water will self-select the bacteria that were present. We presumed that phages that did not find corresponding bacteria would be destroyed. This approach was possible because we have available an inexpensive source of large numbers of isolates of the phages.

We accessed chemical analysis of the spring water in the area that is used for irrigation in order to assess whether the observed impact could be due to chemicals present in the water rather than the phages. The results are given in Table 2.

Details of calcium, magnesium, sodium and potassium were not available. We mixed one liter Ganga water with 9 liters spring water. The chemical composition of the mixed Ganga water is given in Table 3.

Comparison of the spring water and mixed Ganga water indicates nominal difference in the chemical composition except for nitrate. However, the absolute difference in

nitrate of 0.3 mg/L or 3×10^{-4} percent appears insignificant given that concentration from 1 to 3 percent are often used for foliar fertilization of vegetables.¹¹ This insignificant difference in the chemical composition of the two waters suggests that the impact observed may be due to the large numbers of isolates of phages present in the Ganga water.

We administered this diluted water thrice weekly to three crops—cabbage, coriander and spinach—in the author's kitchen garden located at village Lakshmoli, District Tehri, Uttarakhand, India.

The crops had already been planted or sown in January and February 2021. The treatment Mixed Ganga water was started in April 2021. The existing plots were divided into two parts with one part being sprayed with mixed Ganga water and the other part being sprayed with spring water. Spring water is available aplenty in the author's garden and used for irrigation regularly.

3. Results

3.1. Cabbage

The cabbage flowers were harvested as they began to split. The date and weight was noted. It was found that the flowers sprayed with mixed Ganga water matured at a median 8 days later.(Table 4) There was no difference in the median weight. However, the average weight was higher by 6.1 percent. We generally present the results in median which is considered to be more robust.¹² In this case we report averages because the median shows no difference.

3.2. Coriander

The Mixed Ganga water group gave 40 percent higher yield.(Table 5) It may be noted that the number of plants was less by 37 percent. Hence lesser numbers of plants may also have contributed to the higher yield.

3.3. Spinach

The sowing had been done by broadcast method in February 2021. The excess plants were weeded and total number reduced to 21 numbers in early April 2021. The plants were harvested when they began to flower. The number of leaves of more than 2 inch length were counted and the length was individually measured. The "median length of the leaves" was calculated for each plant separately. Then, the median of "median length of the leaves" was calculated.

The numbers of leaves in each group having length of 2 inches or more was noted. The median numbers of leaves for each of the two groups was calculated.

The median length of leaves multiplied by median number of leaves gives us the median yield of leaves in terms of total length. The plants sprayed with mixed Ganga water gave 36.4 percent more numbers of leaves and 8.3 percent higher length of the leaves as shown in last row of

Table 1: Fecal coliform count (Most probable number) in Ganga water, Rudra Prayag.

Hours	0	72	96	120	216	240	264	288
Fecal Coliform Count	440	720	250	150	100	40	80	0

Table 2: Chemical analysis of spring water at four sites in Srinagar.¹⁰

S. No.	Parameter [unit]	Kothar Dhara	Sweet Bridge	Bhola Mahadev	Barkot	Average
1	Alkalinity [mg/L]	190	170	180	250	197.5
2	Acidity [mg/L]	85	57	115	80	84.2
3	D.O.[mg/L]	2	2.80	1.20	6	3
4	B.OD.[mg/L]	0.80	1.40	0.40	1.20	0.95
5	C.O.D [mg/L]	Not Tested	Not Tested	Not Tested	Not Tested	Not Tested
6	Nitrate [mg/L]	0.50	0.30	Not Detected	0.29	0.27
7	Chloride[mg/L]	35.50	15.62	36.92	12.78	25.20
8	Carbon di oxide [mg/L]	48.40	35.20	74.80	39.60	49.5
9	Total hardness[mg/L]	188	147	242	254	207.8
10	pH	6.98	7.33	7.40	7.40	7.27

Table 3: Chemical analysis of Ganga water, Spring water and mixed Ganga water

S. No.	Parameter [Unit]	Ganga water ⁹	Spring water average [Table 2] (Sprayed on Control Plots)	10% Ganga water (+) 90% spring water (Sprayed on Target Plots)	Difference (Percent) Spring water and mixedt ganga water
1	Alkalinity [mg/L]	52	197.5	183.0	(-) 7.3
2	Acidity [mg/L]	Not detected	84.2	75.7	(-) 10.1
3	Dissolved oxygen[mg/L]	6.9	3	3.4	(+) 13.3
4	B.O.D[mg/L]	0	0.95	0.85	(-) 10.5
5	C.O.D [mg/L]	46	Not tested	Not Applicable	Not Applicable
6	Nitrate [mg/L]	3.32	0.27	0.57	(+) 111.1
7	Chloride [mg/L]	12.7	25.2	24.0	(-) 4.7
8	Carbon-di- oxide [mg/L]	Not detected	49.5	44.5	(-) 10.1
9	Total hardness[mg/L]	81	207.7	194.4	(-) 6.4
10	pH	7.6	7.2	7.2	0
11	Calcium[mg/L]	17.6	Not tested	Not Applicable	Not Applicable
12	Magnesium [mg/L]	7.8	Not tested	Not Applicable	Not Applicable
13	Sodium [mg/L]	17.5	Not tested	Not Applicable	Not Applicable
14	Potassium [mg/L]	4	Not tested	Not Applicable	Not Applicable
15	Total dissolved oxygen[mg/L]	81	Not tested	Not Applicable	Not Applicable

Table 4: Cabbage: Effect of Ganga water

S. No.	Group	Number of Days to Harvest, Median (Average)	Weight, Median (Average), Grams	Number of Plants	Size of Plot (Square Feet)
1	Spring water	163 (160)	750 (767)	17	39.5
2	Mixed Ganga water	171 (169)	750 (814)	17	40.8
3	Change, Median (percent)	(+) 8 Days, (+) 4.9%	Nil, (+) 6.1%	Nil	(+) 1.3 Square Feet or (+) 3.3%

Table 5: Coriander: Effect of Ganga water

S. No.	Group	Yield, Grams	Number of Plants	Size of Plot (Square Feet)
1	Spring water	10	232	23.1
2	Mixed Ganga water	14	146	22.0
3	Change, Median (percent)	(+) 4 grams (+) 40%	(-) 86 numbers (-) 37.1%	(-) 1.1 square feet (-) 4.8%

Table 6: Spinach: Effect of Ganga water

S. No.	Group	Number of Leaves, Median (Average)	Length of leaves, Median of Median (Average of Median), inches	Median Number of Leaves x Median Length, inches	Number of Plants	Size of Plot (Square Feet)
1	Spring water	11 (11.7)	3.00 (3.20)	33.0	21	11.7
2	Mixed Ganga water	15 (17.6)	3.25 (3.44)	48.7	21	12.1
3	Change, Median, percent	(+) 4 Numbers (+) 36.4%	(+) 0.25 Inches (+) 8.3%	(+) 15.7 Inches (+) 47.6%	Nil	(+) 0.4 (+) 3.4%

Table 7: Summary results

S.No.	Crop	Unit	Result	Confounding Contributors
1.	Cabbage	Average weight, grams	(+) 6.1%	Plot size greater by 3.4 percent.
2.	Coriander	Weight, grams	(+) 40%	Number of plants was less by 37.1 percent.
3.	Spinach	Median Number of Leaves x Median Length, inches	(+) 47.6%	Plot size greater by 3.4 percent.

Table 6. The number of leaves multiplied with the length of leaves gave 47.6 percent higher yield. The plot size for the Mixed Ganga water group was greater by 3.4 percent. We ignore this.

3.4. Implications for human immunomodulation

Our study is limited to one year. However, we report the results considering that the world is facing an unprecedented Corona pandemic and our results may provide a possible pathway for the immunomodulating the human response to bacterial infections. Rodrigo A. Valverde, Professor in the Department of Plant Pathology and Crop Physiology at the Louisiana State University has drawn attention to the similarities between the viral infections in plants and in the infections of humans by Covid-19. The tobacco mosaic virus, for example, is controlled by the field and greenhouse workers by disinfecting their hands, tools and equipment. Plants are often protected by “changing the environment” like using reflexive mulches, covering plants with fabrics, spraying with oils and growing in greenhouses. These are similar to the use of disinfectants, wearing masks and home isolation to control the spread of Covid-19.¹³ Following this lead we draw attention to the possibility of using the natural phage cocktail present in the Ganga waters for improving immunity of human beings.

The ancient Indian medical text by Vagbhat says: “Water of Ganga is enlivening, satiating, good for heart, calming, stimulates intellect, clean, of imperceptible taste, cold, light and similar to nectar”.¹⁴ Another ancient text Rajnighantu says, “Ganga Jal is pure, tasty, digestive and very meditative as well as its consumption and a dip in it makes a person sin free. It quenches your thirst as well as greediness and destroys the evilness in you. Ganga Jal has millions of benefits and is very effective in many diseases”.¹⁵

Another recent research conducted by Malaria Research Center, New Delhi, shows that mosquito breeding did not occur in the upper stretches of River Ganga and

also in waters to which Ganga water was added. They concluded that bacteriophages, phytochemicals, metals and biodegradation by other microbes together keep the water clean and pure.¹⁶

A study by Bagdi, Niwani and Jhunjhunwala has reported 70 percent relief from Upper Respiratory Tract infections, 30 to 60% relief in Lower Respiratory Tract infections and some relief in gastro-intestinal diseases in human patients.¹⁷ However, these results were obtained from observational studies without a control sample. We provide confirmation of these results from plants with control samples.

The safety of Ganga water requires consideration before use in humans. The Mean Dissolved Oxygen at the location where we collected the water was 9.6 mg/liter and Mean Biological Oxygen Demand was 1.2 mg/liter. Both these parameters are fit for drinking water. The pH is also reported to be within drinking water criteria of 6.5 to 8.5 though the figures are not given. The problem lies with coliforms that are reported at 9300 MPN per 100 ml against the requirement of <50 for drinking water.¹⁸ We stored the water with sediments. As reported above, we found that the phages adsorbed into the sediments cleaned up the overlying water column of all the coliforms. Hence, the water was safe for drinking.

4. Discussion

The summary results are given Table 7.

The higher plot size for spinach and cabbage by 3.3 and 3.4 percent is small and we feel this can be ignored.

The median number of days to maturity in cabbage is 4.9 percent higher. However, the median yield gives no difference. This may be considered a quirk of data since the average yield shows a parallel 6.1% increase.

The lesser number of coriander plants could have added to the yield and the 40 percent higher yield, therefore, needs to be taken only as a tentative result.

5. Conclusion

This study provides evidence for prophylactic efficacy of natural cocktail of phages in the Ganga river on plants. It points to the possibility of use of Ganga water for immunomodulation in humans.

6. Conflict of Interest

The authors state no conflict of interest.

7. Source of Funding

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