



A Bacterial Consortium and Synthetic Fertilizer Based Biocontrol Approach Against Potato Rot Disease “*Neocosmospora rubicola*”

Muhammad Riaz^{1,2}, Rashid Mahmood³, Mohammed Antar², Naureen Akhtar^{1,4}, Salik Nawaz Khan¹, Muhammad Ashfaq Anjum⁵ and Donald L. Smith^{2*}

¹ Department of Plant Pathology, University of the Punjab, Lahore, Pakistan, ² Department of Plant Science, McGill University, Montreal, QC, Canada, ³ Department of Soil Science, University of the Punjab, Lahore, Pakistan, ⁴ Monash Institute of Pharmaceutical Sciences, Faculty of Pharmacy and Pharmaceutical Sciences, Monash University, Melbourne, VIC, Australia, ⁵ Soil Bacteriology Section, Ayub Agriculture Research Institute, Faisalabad, Pakistan

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*Correspondence:

Donald L. Smith
donald.smith@mcgill.ca

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Conventional management of stem rot disease of potato, caused by *Neocosmospora rubicola*, through fungicide application is an environmentally unfavorable practice that calls for an alternative biocontrol approach. Plant growth-promoting bacteria (PGPB) are known to not only promote plant growth but also control diseases caused by various fungi. The study was designed to evaluate the potential of three strains of PGPB and synthetic fertilizer to manage stem rot of potatoes. In the first experiment, PGPB strains *Azotobacter chroococcum*, *Azospirillum lipoferum*, and *Pseudomonas putida* and their combinations were evaluated on PDA medium against *N. rubicola* using the dual culture technique. All three bacterial strains were found effective in reducing the radial growth of the fungus maximum up to 91%. In the second experiment, in the presence of half and full recommended doses of fertilizer nitrogen (N) and phosphorus (P), the potato growing medium was inoculated with *N. rubicola* alone, and with combinations of *N. rubicola* and PGPB strains (bacterial formulation; BF). *N. rubicola* increased stem and tuber rot, and decreased tuber weight by 11% compared to the control. On the other hand, sole inoculation with BF significantly increased tuber weight. In addition, a combined inoculation of *N. rubicola* and BF, or *N. rubicola* inoculation a week prior to BF inoculation did not affect tuber weight compared to control. However, inoculation of BF a week prior to *N. rubicola*, controlled rot symptoms and increased tuber weight by 32%. An increase in P application favored the PGPB strains in controlling rot in tubers. The interaction effect of fertilizer N with the inoculation combinations was non-significant; however, the main impact of N was to increase rot in tubers and decrease in potato stems. Hence a prerequisite application of PGPB formulation proved to be an effective tool against *N. rubicola* infestation in potatoes.

Keywords: *Neocosmospora rubicola*, potato stem rot, *Azotobacter chroococcum*, *Azospirillum lipoferum*, *Pseudomonas putida*, bacterial formulation, biofertilizer

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important staple crops, and its long-term cultivation faces a significant threat of plant diseases (Dahal et al., 2019). These diseases are frequently managed by conventional agricultural practices, particularly by using various types of fungicides, which have serious environmental concerns (Chandra et al., 2020; My et al., 2021). Furthermore, sole and excessive use of chemical fertilizers to support intensive cropping systems also negatively impacts the environment by causing soil infertility, groundwater contamination, climate change, and imbalance of soil and water ecosystems (Liu et al., 2015; Besser and Hamed, 2021). Nutrient management by integrating inorganic fertilizers, manure, and biofertilizers has beneficial effects over the sole application of chemical fertilizers (Tafesse and Leta, 2019). Plant growth-promoting bacteria (PGPB) from biofertilizers promote plant growth through biological nitrogen fixation, phosphate solubilization, micronutrient solubilization, biosynthesis of phytohormones and other plant growth-promoting substances. Some PGPB also exhibits biocontrol properties and show antagonistic activity against various pathogenic fungi of crop plants (Mohammadi and Sohrabi, 2012; Pellegrini et al., 2020).

The rhizosphere is the soil around plant roots, where microbes mediate complex processes, and it is a hotspot for microbial community interactions (Paterson and Mwfulirwa, 2021). The plethora of microbial communities found in the rhizosphere comprises bacteria that are hostile to other microbes (Smith et al., 2015; Antar et al., 2021). These antagonistic bacteria interfere with pathogen growth and act as predators for other microbes (Chandra et al., 2020). The antagonistic bacteria play a vital role in the rhizospheric zone as a biocontrol agent and suppress phytopathogenic fungi (Anith et al., 2021). Several PGPB, in addition to their influence on plant growth and function through phytohormone synthesis, have been explored for their antifungal potential against many phytopathogens.

Most potential and widely reported PGPB genera associated with solanaceous crops to include *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Burkholderia*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Serratia*, and endophytic bacteria such as *Bacillus*, *Erwinia*, and *Xanthomonas* (Smith et al., 2015; Çevik and Ogutcu, 2020; Kumari et al., 2021). It has been reported that PGPB such as *Azotobacter chroococcum*, *Azospirillum lipoferum*, and *Pseudomonas putida* have the ability to produce siderophores, indole-3-acetic acid (IAA), hydrogen cyanide (HCN), and solubilize phosphate, act as biological control agent and enhance plant growth and development of potato (Sepehrnush et al., 2018). *Azotobacter chroococcum* naturally exists in the rhizosphere of various crops, releases some plant growth hormones and is capable of nitrogen fixation at about 20 kg N ha⁻¹ year⁻¹ (Kumar et al., 2018; Song et al., 2021). Maheshwari et al. (2012) demonstrated that the strain TRA2 of *A. chroococcum*, an isolate of the wheat rhizosphere, showed strong antagonistic activity against root rot fungus *Macrophomina phaseolina* and *Fusarium oxysporum* and improved plant growth of wheat. *Azospirillum lipoferum*, a free-living nitrogen-fixing bacterium, also produces phytohormones

to promote the growth of inoculated plants, particularly under stress conditions (Czarnes et al., 2020; Batabyal, 2021). *Pseudomonas putida*, a Gram-negative bacterium, benefits crop plants by acting as a phosphate solubilizer and biocontrol agent (Sun et al., 2017; Takishita et al., 2021). The bacterium has a Type VI Secretion System (T6SS) to kill phytopathogens, which is important in its biocontrol portfolio (Borrero De Acuña and Bernal, 2021). It was found effective for a number of destructive plant pathogens like *F. oxysporum* in tomato, *Rhizoctonia solani* and *Pectobacterium atrosepticum* in potato and *Sclerotinia sclerotiorum* in lettuce (Bernal et al., 2017; Durán et al., 2021). Siderophores produced by *P. putida* can also improve the iron nutrition of crop plants (Meliani et al., 2017). Due to their various beneficial characteristics, *A. chroococcum*, *A. lipoferum*, and *P. putida* are used as PGPB in biofertilizers.

Neocosmospora rubicola is a filamentous fungus in the family Nectriaceae. It causes stem rot of Pitaya (*Hylocereus costaricensis*) in China (Zheng et al., 2018), root rot of *Glycyrrhiza uralensis* in Korea (Kim et al., 2017), and stem rot of potatoes in Pakistan (Riaz et al., 2020). Under laboratory conditions, the pathogen grows optimally at 26°C and near-neutral pH (Riaz et al., 2020). This favorable to the pathogen temperature (26°C) prevails in many potato growing areas of the world. An occurrence frequency percentage of *N. rubicola* was found to vary from 18 to 89% during a survey of potato fields of seven districts of Punjab, Pakistan (unpublished data).

The present study was designed to determine if PGPB *A. chroococcum*, *A. lipoferum*, and *P. putida* have the potential to suppress *N. rubicola* and its infestation to potato plants. The performance of the PGPB was evaluated against the pathogen in interaction with nitrogen (N) and phosphorus (P) nutrition of potato plants.

MATERIALS AND METHODS

Isolation and Identification of *Neocosmospora rubicola*

Neocosmospora rubicola was isolated from rotting potato stems collected during a survey of potato fields at Kot Radha Kishan (31°10'21N, 74°5'59E), district Kasur, Punjab, Pakistan. For fungal isolation, the infected stem was sterilized with 10% sodium hypochlorite (NaClO) for 30 s, and pieces (2 mm²) of the stem were incubated on potato dextrose agar (PDA) followed by purification through single spore inoculation (Muhammad et al., 2018; Zheng et al., 2018). Morphological identification of the fungus was conducted by the First Fungal Culture Bank of Pakistan (FCBP) as *Neocosmospora* genus. For species-level identification, genomic DNA of the fungus was isolated (Shafique et al., 2019) to perform PCR using two primers: Internal Transcribed Spacer (ITS) of rDNA and partial beta-tubulin gene (Park et al., 2012). PCR products were sequenced by SolGent (Daejeon, Korea). The acquired nucleotide sequences were deposited at GenBank (rDNA-ITS regions ID: MG976818 and partial beta-tubulin gene ID: MH016281) and differentiated from the pool of nucleotide sequences in GenBank by BLAST. BLAST results indicated that the present fungal

strain's rDNA-ITS and atrial beta-tubulin genes were 100% similar to their corresponding sequences of *N. rubicola* strain CBS 320.73 submitted under the accession numbers KM231799 and KM232061. A pathogenicity test of *N. rubicola* as a causal agent of potato stem rot was performed. Further details of isolation, identification and pathogenicity test are described in our previous publication (Riaz et al., 2020).

In-vitro Evaluation of PGPB Strains Against *N. rubicola*

Pure cultures of three PGPB, namely *A. chroococcum*, *A. lipoferum*, and *P. putida*, were obtained from the Bacteriology section of Ayub Agriculture Research Institute (AARI) Faisalabad, Pakistan. This institute uses these PGPB to form a biofertilizer commercially available in the local market with the trade name of Faslon ka Jraseemi Tekka (FKJT). The inhibition potential of PGPB strains against *N. rubicola* was evaluated using *in-vitro* dual culture techniques (Khruengsai et al., 2021). In this experiment, a solidified PDA Petri plate was an experimental replicate/unit. One 4 mm diameter mycelial disc from the margin of *N. rubicola* 7 days old culture was used to inoculate each agar-solidified PDA Petri plate by making the same size hole at the PDA center in the plate. The treatment plan was comprised of bacterial strains *viz.* *A. chroococcum* (Ac), *A. lipoferum* (Al), *P. putida* (Pp), Ac + Al, Ac + Pp, Al + Pp and Ac + Al + Pp, which were streaked 2 cm away from the central mycelial disc. The streaking was performed by making about 2 cm long single lines on three sides of the central disc using a sterilized wire loop. The experiment was conducted according to a completely randomized design (CRD), where each treatment was replicated three times. Petri plates were then incubated at 26°C for 5 days. After incubation, the colony diameter of the fungal growth in each replicate was measured from the same three sides where streaking was performed, and averages were used in subsequent statistical analyses.

Evaluation of Synthetic Fertilizer and Inoculation With *N. rubicola* and PGPB Formulation on Growth and Yield of Potato

The experiment was conducted in plastic pots, 30 cm in height and 30 cm in diameter, after filling with 12 kg of loamy soil (Table 1). The experimental design was three times replicated CRD with N application rate, P application rate, and inoculation combination (IC) of *N. rubicola* (Nr) and/or PGP bacterial formulation (BF) as three experimental factors. The plan was comprised of half and full recommended rates of N (125 and 250 kg N ha⁻¹), half and full recommended rates of P (60 and 120 kg P₂O₅ ha⁻¹), and six IC's of Nr and BF, i.e., no inoculation,

and inoculation with Nr, BF, Nr + BF, Nr followed by BF with a week interval and BF followed by Nr with a week interval. The recommended rates of N and P for potatoes were obtained from the literature published by Pakistan Agriculture Research Council (PARC) for the farming community (PARC-Pakistan Agriculture Research Council, 2002).

Each experimental unit was a pot in which three seed potatoes (Kuroda variety of Agrico, UK) of uniform size and the same number of buds were cultivated. Prior to planting, potassium sulfate at the rate of 125 kg K₂O ha⁻¹, whole P as single super phosphate (SSP) and half of the N as urea was applied to pots soil. The remaining half dose of N was applied at 30 days after planting (DaP) of seed potatoes.

The inoculation applications were started at 30 DaP. For pathogen inoculation, a spore suspension of *N. rubicola* was prepared in Tween-80 solution (0.9 % NaCl and 0.1% Tween 80). The spore count in the suspension was estimated by the hemocytometer as 5.35 × 10⁶ mL⁻¹. The suspension was applied to pot soil at 10 mL per pot followed by light irrigation to assure the spore penetration to the root zone. For BF preparation, FKJT biofertilizer was obtained from the Bacteriology section of AARI, which they claimed to have 1.2 × 10⁶ colony-forming units (CFU) of *A. chroococcum*, *A. lipoferum*, *P. putida* on 1 g of a carrier material (Rafique et al., 2018). For a single pot, 5 g FKJT was suspended in 20 mL of 10% sugar solution and applied over the soil surface, followed by a light irrigation. In this way, the total CFU added to a pot was 6 × 10⁶.

Analysis of Changes in the Growth Parameters and Leaf Nutrients of the Potato

At 65 DaP, a leaf, 4th from the growing tip, was sampled from each plant and analyzed for nitrate, potassium and phosphorous. Nitrate was water-extracted from the fresh leaf tissue and estimated through nitration of salicylic acid in the presence of concentrated sulfuric acid followed by optical density measurement at 410 nm (Cataldo et al., 1975). Dried leaves were digested in a 2:1 mixture of nitric acid and perchloric acid for P and K determinations. Ammonium heptamolybdate-ammonium vanadate and flame photometer methods were used for P and K estimation from the digests, respectively (Jones et al., 1991).

At 70 DaP, variables such as plant height, the total number of stems per pot, the number of stems with rot symptoms per pot and average stem width per pot were estimated. At maturity, the plants were harvested, tubers were dug up, and the total number of tubers per pot, the number of tubers with rot symptoms per pot and potato yield per pot were determined.

The pot experiments were conducted in three growing seasons under a rain shelter to block natural rainfall. The rain shelter was generally closed throughout growing seasons and opened only during rainfalls. The experimental results were averaged over growing seasons before applying statistical analysis. The data of all the variables were subjected to analysis of variance, and means were compared with Tukey's HSD test. The Pearson correlation between various analytical and growth variables was estimated by using Statistix 8.1 computer software.

TABLE 1 | Chemical properties of loamy soil used in the experiment.

Soil type	Chemical analysis				
	pH	CEC (dS m ⁻¹)	%O.M	P (ppm)	K (ppm)
Loam	7.8	1.5	0.69	14.3	302

RESULTS

Antifungal Activity of Selected PGPB Against *N. rubicola* Radial Growth

The dual culture technique was followed to evaluate three strains of PGPB against *N. rubicola* in Petri-plates. After 5 days of inoculation, the effect of PGPB on the radial growth of the fungus was significant. Maximum radial growth was observed in the treatment where the fungus was inoculated alone on PDA (control). Dual inoculation of “*N. rubicola* + *A. chroococcum* + *P. putida*” and “*N. rubicola* + *A. chroococcum* + *A. lipoferum* + *P. putida*” reduced radial growth of the fungus up to 91% in comparison to the control (Figure 1). Inoculating *N. rubicola*

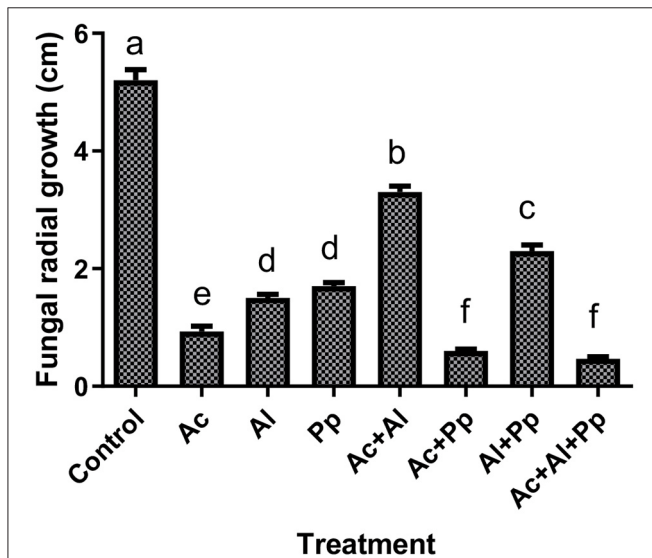


FIGURE 1 | Fungal radial growth of *Neocosmospora rubicola* dual cultured with *Azotobacter chroococcum* (Ac), *Azospirillum lipoferum* (Al), and *Pseudomonas putida* (Pp) bacterial strains (PGPB). Bars, means \pm standard errors, sharing common letter(s) do not differ significantly at $p < 0.05$ by Tukey's HSD test.

with a single PGPB of *A. chroococcum*, *A. lipoferum*, or *P. putida* caused radial growth inhibition up to 82, 71 and 67%, respectively (Figure 1).

Effect of Synthetic Fertilizer and Inoculation With *N. rubicola* and PGPB Formulation on Growth and Yield of Potato

The significance levels (p values) of main effects and interactions in the analysis of variance are presented in Table 2. A highly significant difference was observed among fertilizer N levels for all variables and fertilizer P for the majority of the variables except the rot variables, i.e. the number of rotten stems and number of rotten tubers. In addition, there were highly significant differences among inoculation combinations (IC) for all measured variables.

Except for rotten stems (%) and leaf nitrate, N \times P interaction was non-significant for all parameters. There was no N \times IC interaction for the number of stems, tubers, and tuber yield. However, the interaction existed for rotten stems (%), rotten tubers (%), plant height and stem diameter. There was no P \times IC interaction for most variables except leaf nitrate, leaf phosphorus and the number of rotten tubers. A three-way interaction for N \times P \times IC was found significant only for leaf nitrate contents (Table 2).

The means of variables measured for their response to N and P fertilizers are presented in Tables 3, 4, respectively. An increase in N from 125 to 250 kg ha⁻¹ decreased stem rot and increased the number, diameter, and height of stems by 10–14%. ‘This increment in N dose increased potato yield by 4% and tuber number by 6% and caused rot in 23% more tubers. Leaf nitrate contents of plants treated with 250 kg N ha⁻¹ were about 122% more than those supplied with a half dose of N (125 kg ha⁻¹). Doubling the N dose also increased K and P in potato leaves by 3 and 15%, respectively (Table 3).

An increase in P-fertilizer from 60 to 120 kg P₂O₅ ha⁻¹ significantly increased P, nitrate and K in potato leaves by 40, 22, and 11%, respectively. In response to 120 kg P₂O₅ ha⁻¹, potato stems were 2–4% greater in number, height, and diameter than those at 60 kg P₂O₅ ha⁻¹. A similar response was noted for the

TABLE 2 | Probability levels of the variance ratio from analysis of variance of variables scored on two rates of fertilizer nitrogen (N) and fertilizer phosphorus (P), and six inoculation combinations (IC).

Parameters	N	P	IC	N \times P	N \times IC	P \times IC	N \times P \times IC
Plant height	<0.001	0.014	<0.001	0.133	0.001	0.318	0.458
Stem diameter	<0.001	<0.001	<0.001	0.265	0.009	0.338	0.368
No. of stems	<0.001	0.027	<0.001	1.000	0.065	0.297	0.948
No. of rotten stems	<0.001	0.064	<0.001	0.064	0.060	0.119	0.203
No. of tubers	<0.001	0.003	<0.001	0.751	0.142	0.980	0.944
No. of rotten tubers	<0.001	0.102	<0.001	0.188	0.204	0.038	0.225
Tuber yield	<0.001	<0.001	<0.001	0.330	0.375	0.907	0.220
Leaf nitrate	<0.001	<0.001	<0.001	0.009	0.002	<0.001	0.020
Leaf phosphorus	<0.001	<0.001	<0.001	0.241	0.294	0.002	0.560
Leaf potassium	<0.001	<0.001	<0.001	0.467	0.830	0.320	0.846

The bold values indicate the significance levels (p values) of main effects and interactions in the analysis of variance.

TABLE 3 | Variations in potato plant growth, yield, and nutrient variables in response to nitrogen fertilizer.

Parameters	Nitrogen fertilizer		HSD ($p < 0.05$)
	Half of recommended (125 kg ha ⁻¹)	Recommended (250 kg ha ⁻¹)	
Plant height (cm)	15.2 b*	16.7 a	0.26
Stem diameter (mm)	6.4 b	7.3 a	0.13
No. of stems pot ⁻¹	12.6 b	14.1 a	0.44
No. of rotted stems pot ⁻¹	3.2 a	2.7 b	0.34
No. of tubers pot ⁻¹	19.5 b	20.8 a	0.53
No. of rotted tubers pot ⁻¹	2.6 b	3.2 a	0.33
Tuber yield (kg pot ⁻¹)	0.71 b	0.74 a	0.01
Leaf nitrate (ppm)	5.3 b	11.8 a	0.77
Leaf phosphorus (ppm)	19.7 b	22.8 a	1.04
Leaf potassium (%)	0.30 b	0.31 a	0.006

*Means sharing common letter(s) in a row do not differ significantly at $p < 0.05$ by Tukey's HSD Test.

TABLE 4 | Variations in potato plant growth, yield, and nutrient variables in response to phosphorus fertilizer.

Parameters	Fertilizer phosphorus		HSD ($p < 0.05$)
	Half of recommended (60 kg ha ⁻¹)	Recommended (120 kg ha ⁻¹)	
Plant height (cm)	15.8 b*	16.1 a	0.26
Stem diameter (mm)	6.7 b	7.0 a	0.13
No. of stems pot ⁻¹	13.1 b	13.6 a	0.44
No. of rotted stems pot ⁻¹	3.1 a	2.8 a	0.32
No. of tubers pot ⁻¹	19.8 b	20.6 a	0.53
No. of rotted tubers pot ⁻¹	2.8 a	3.0 a	0.33
Tuber yield (kg pot ⁻¹)	0.70 b	0.72 a	0.01
Leaf nitrate (ppm)	7.7 b	9.4 a	0.77
Leaf phosphorus (ppm)	17.7 b	24.8 a	1.04
Leaf potassium (%)	0.27 b	0.30 a	0.006

*Means sharing common letter(s) in a row do not differ significantly at $p < 0.05$ by Tukey's HSD Test.

number of tubers per pot and tuber yield. However, P addition had no significant effect on rot parameters, i.e., number of rotten stems and rotten tubers (Table 4).

The effect of inoculation of *N. rubicola* and BF on growth, yield and rot of tubers is presented in Table 5. Sole inoculation of *N. rubicola* significantly reduced plant height and number of stems per pot compared to the control. Maximum height and the number of stems were recorded in potato plants where BF was applied alone or 1 week prior to the inoculation of *N. rubicola*. Application of BF 1 week after inoculation of *N. rubicola* did not increase plants' height and the number of stems compared to control. However, a combined inoculation of *N. rubicola* and BF increased plant height by 10% compared to that of non-inoculated, and 27% compared to that inoculated with *N. rubicola* alone (Table 5).

The minimum number of rotten stems and tubers were observed in the absence of *N. rubicola* inoculation, followed by BF application a week prior to the pathogen inoculation and a combined inoculation of *N. rubicola* and BF. Sole inoculation of *N. rubicola*, or its inoculation a week prior to BF, caused the maximum number of rotten stems and tubers (Table 5).

Compared to the control, tuber yield decreased with *N. rubicola* inoculation by 11% and increased with BF by 41%. Inoculation of *N. rubicola* a week prior to or combined with BF did not affect tuber yield compared to the control. The pathogen had no effects on tuber yield when it was inoculated a week prior to BF application. Bacterial formulation increased tuber yield by 32% in comparison to the control (Table 5).

N. rubicola significantly decreased, and BF increased nitrate, P and K contents in potato leaves. These contents were maximum with sole BF application, followed by BF application a week prior to *N. rubicola*, and a combined application of both. Leaf nitrate, P and K were found to be minimal with *N. rubicola* alone, which were similar to when *N. rubicola* was applied a week prior to the BF (Table 5).

N, P, and K concentrations were negatively correlated with the number of rotten stems and tubers (Table 6). However, a highly significant and positive correlation ($r = 0.91$) was observed between the number of rotten stems and tubers. In the same way, there were significant positive correlations between nitrate, P and K concentrations in potato leaves (Table 6).

The interaction effect of treatments and P fertilizer on the number of rotten tubers is presented in Figure 2. Tuber rot due to sole inoculation of *N. rubicola* was not influenced by P fertilizer doses. However, an increase in P fertilizer dose along with BF application significantly reduced the number of rotten tubers in the treatments where the pathogen was applied.

DISCUSSION

The radial growth-suppression of *N. rubicola* in the PDA medium seemed to be related to the production of anti-fungal compounds by the PGPB strains. These compounds might be hydrocyanic acid (HCN) produced by *A. chroococcum* (Gopalakrishnan et al., 2011), salicylic acid, 2,3-dihydroxybenzoic acid (DHBA), and 3,5-DHBA conjugated with threonine and lysine by *A. lipoferum* (Shah et al., 1992), and volatile organics belonging to pyrazines such as 2-methylpyrazine and 2-ethyl-3,6-dimethylpyrazine by *P. putida* (Patel et al., 2021). Owing to the production of these compounds, and strengthening the plants by phosphate solubilization, nitrogen fixation, and producing siderophores, indole acetic acid (IAA), gibberellins, cytokinins, and vitamins (Gopalakrishnan et al., 2011; Seddigui Kiasari et al., 2018; Mehmood et al., 2021), these PGPB are already reported to have anti-fungal activity against common scabs of potato (Sowanprecha and Rerngsamran, 2018), late blight of potato (Hultberg et al., 2010), Fusarium wilt of radish and some other plant diseases caused by soil-borne fungi (Weller, 2007).

Plants grown under the recommended dose of fertilizers showed better growth and yield than those supplied with half-dose, solely due to nutrient sufficiency and behavioral response

TABLE 5 | Variations in potato plants' growth, yield, and nutrient variables in response to soil inoculation with various combinations of *Neocosmospora rubicola* (Nr) and the bacterial formulation (BF).

Parameters	Control	Nr	BF	Nr+BF	BF followed Nr	Nr followed BF	HSD ($p < 0.05$)
Plant height (cm)	14.9 c*	12.9 d	19.5 a	16.4 b	14.7 c	18.3 a	0.65
Stem diameter (mm)	6.8 c	5.6 d	8.3 a	6.6 c	5.9 d	7.8 b	0.33
No. of stems pot ⁻¹	13.4 cd	10.3 e	15.6 a	13.9 bc	12.3 d	14.6 ab	1.13
No. of rotted stems pot ⁻¹	0.5 d	7.1 a	0.6 d	5.4 b	7.3 a	2.08 c	0.75
No. of tubers pot ⁻¹	20.5 b	15.5 d	23.6 a	20 bc	18.4 c	22.5 d	1.35
No. of rotted tubers pot ⁻¹	0.33 c	6.0 a	0.33 c	4.4 b	5.2 ab	1.1 c	0.86
Tuber yield (kg pot ⁻¹)	0.61 d	0.54 f	0.86 a	0.75 d	0.61 d	0.81 b	0.027
Leaf nitrate (ppm)	10.6 b	5.8 cd	15.9 a	7.57 c	4.43 d	10.6 b	1.97
Leaf phosphorus (ppm)	20.7 b	10.9 d	30.2 a	21.3 b	16.3 c	28.1 a	2.65
Leaf potassium (%)	0.28 b	0.24 d	0.34 a	0.29 b	0.26 c	0.31 a	0.02

*Means sharing common letter(s) in a row do not differ significantly at $p < 0.05$ by Tukey's HSD test.

TABLE 6 | Correlation coefficients (Pearson) among leaf nutrient status and various rotting variables of potato.

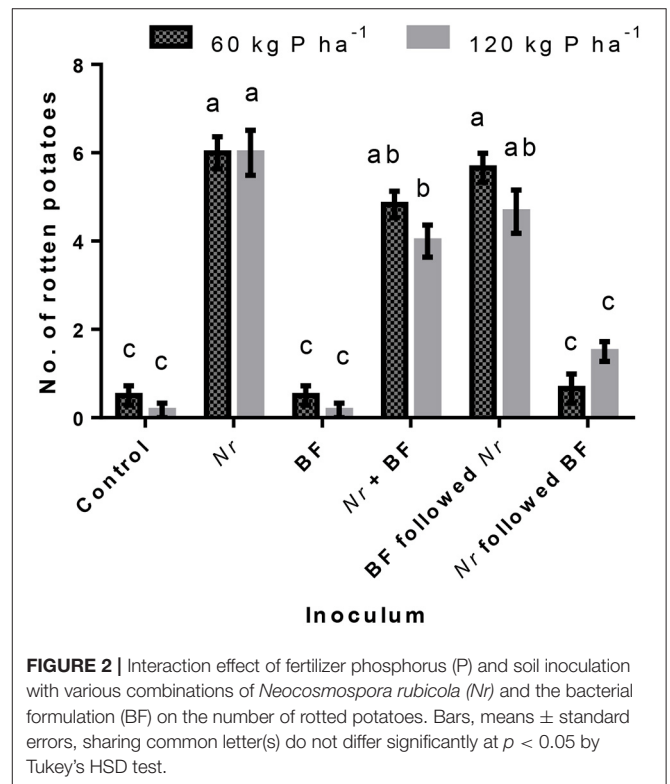
Parameters	Leaf nitrate	Leaf P	Leaf K	Rotted stems
Leaf P	0.59**			
Leaf K	0.32*	0.46**		
Rotted stems	-0.60**	-0.66**	-0.26*	
Rotted potatoes	-0.57*	-0.70**	-0.29*	0.91**

**Significant at $p < 0.001$, *Significant at $p < 0.05$.

of nutrient-deficient plants to nutrient applications (Baligar et al., 2001). Nitrogen and phosphorus applications enhanced their synergistic uptake along with potassium, as indicated by a significant positive correlation between nitrogen, phosphorus and potassium concentrations in potato leaves. This synergistic behavior of N, P, and K is well-documented in the literature for several crop plants (Binder et al., 2000; Zhang et al., 2010).

Soil inoculation of *N. rubicola* significantly reduced potato growth and yield by rotting the stems and tubers. This reduction in growth and yield variables of potatoes was caused by the disintegration of vascular bundles, which restricted the ability of potatoes to uptake and distributed nutrients and water (Chavarria and dos Santos, 2012). The detrimental effects of *N. rubicola* on nutrient uptake were also evident from the negative correlation of N, P, and K concentrations in potato leaves with the number of rotten stems and tubers (Table 6). The report of *N. rubicola* as a causal agent of stem rot of potatoes is relatively recent (Riaz et al., 2020). However, it was previously found to cause rots and severe yield reductions in *Hylocereus undatus* (Zheng et al., 2018), *Glycyrrhiza uralensis* (Kim et al., 2017), and *Pyrus* spp. (An et al., 2016).

The application of BF increased potato growth and yield possibly due to (i) nitrogen fixation by *A. chroococcum* and *A. lipoferum*, particularly more important in nitrogen-deficient plants (Cassán and Diaz-Zorita, 2016; Gothandapani et al., 2017), (ii) phosphate solubilization by *P. putida* (Sowanpreecha and Rerngsamran, 2018), (iii) production of

**FIGURE 2** | Interaction effect of fertilizer phosphorus (P) and soil inoculation with various combinations of *Neocosmospora rubicola* (Nr) and the bacterial formulation (BF) on the number of rotten potatoes. Bars, means \pm standard errors, sharing common letter(s) do not differ significantly at $p < 0.05$ by Tukey's HSD test.

growth promoters/hormones in the rhizosphere (Gothandapani et al., 2017), and (iv) biological suppression of *N. rubicola* in infested soil to minimize rot incidence and severity (Sowanpreecha and Rerngsamran, 2018). The BF-induced N fixation and P solubilization were evident from the increase in N and P concentrations in potato plants.

Application of the BF a week prior to *N. rubicola* inoculation nullified the effect of pathogen and increased potato growth and yield compared to the control. In contrast, *N. rubicola* inoculation a week prior to BF masked the positive impact of BF under detrimental pathogenic effects. However, a combined

inoculation of the pathogen and the BF neither enhanced nor reduced potato yield. These responses indicated that the BF could potentially encounter detrimental effects of *N. rubicola* on potato plants, mainly when applied prior to disease occurrence. Furthermore, the BF strengthened plants against disease incident and severity through better and balanced nutrition and by providing plant growth hormones (Ouhaibi-Ben Abdeljalil et al., 2016).

Nitrogen decreased the number of rotten stems and increased the number of rotten potatoes. This behavior of the pathogen should be further explored. However, it can be hypothesized that N-induced rapid growth of potato stems better tolerated destructive effects of the fungal pathogen (Mackenzie, 1981). In contrast, high N concentration reduced tuber-specific gravity to promote tuber rot (Wilson et al., 2019).

Increasing fertilizer-P in the presence of BF significantly reduced the number of rotten potatoes associated with treatments where the pathogen influence was significant. This indicated that inorganic P could have a synergistic impact on bacterial strains of the BF, particularly on *A. lipoferum* (Eftekhari et al., 2012), which suppressed the pathogen through its biocontrol potential (Mazhar et al., 2016).

CONCLUSION

Bacterial formulation (BF) containing strains of *A. chroococcum*, *A. lipoferum* and *P. putida* has the potential to increase the tuber yield of potato in healthy soil as well as in the soil infested with *N. rubicola*. Phosphorus applications along with the BF can improve the working efficiency of the bacterial strains. However, nitrogen fertilizer can favor *N. rubicola* infestation

without interfering with the working of the strains. To control *N. rubicola* infestation, with maximum tuber yield benefits, a pre-application of the biofertilizer is a better option. However, this study calls for future research to verify experimental results in different types of soils, in open field crops and under different pedoclimatic conditions.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: NCBI [accession: KM231799 and KM232061].

AUTHOR CONTRIBUTIONS

MR: conceptualized the study, formal analysis, methodology, and writing of original draft/manuscript. RM: designing of survey format and compilation and interpretation of data. SK: review and editing, supervision, and technical input. NA: supervised, reviewed, and edited the manuscript and provided technical guidelines during the study. MAA: field visits for collection of data and provided the biofertilizer. MA: technical input and edited the manuscript. DS: review and editing, supervision, and technical input. All authors contributed to the article and approved the submitted version.

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