Biocontrol of strawberry diseases using antagonistic bacteria

ANURADHA BISHNOI[1*], POONAM SAINI[2], RAKESH KUMAR[3] and MEHAK NAGORA[1]

[1]Regional Research Station, [3]College of Agriculture, CCS HAU, Bawal 123501 Haryana, India
[2]Regional Research Station, CCS HAU, Buria, Yamuna Nagar 135101 Haryana, India
*Email for correspondence: anuradhabishnoi@hau.ac.in

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ABSTRACT

This two-year study investigated the effect of antagonistic rhizospheric bacteria on the incidence of gray mold (*Botrytis cinerea*) and anthracnose (*Colletotrichum* spp) in strawberry fruits. The experiment, conducted under a completely randomized design, evaluated 12 bacterial strains and two fertilizer controls on the Chandler strawberry variety. The results showed that specific bacterial strains significantly reduced the incidence of both diseases. For gray mold, treatments with ABS5:RCA7, ABS4:HCA61 and APS4:HMR25 consistently provided the lowest disease incidence compared to control groups. Similarly, for anthracnose, the bacterial strains ABS4:HCA61 and ABS5:RCA7 proved to be the most effective. The study's findings demonstrate that these antagonistic bacteria are highly effective biocontrol agents, offering a promising, eco-friendly alternative to conventional chemical treatments for managing major strawberry diseases.

Keywords: Strawberry; antagonistic bacteria; biocontrol; gray mold; anthracnose; *Botrytis cinerea*; *Colletotrichum* spp

INTRODUCTION

Strawberry (*Fragaria* × *ananassa* Duch), belonging to the family Rosaceae, is known as one of the most attractive, delicious and refreshing fruits of the world and occupies an important place among the orchard plants (Anuradha et al 2018). The cultivated strawberry is an octaploid (2n = 8x = 56) stoloniferous perennial herb (Debnath and Teixiera da Silva 2007). It is considered as a nutrient rich fruit having many health benefits due to antioxidant, antimutagenic and anticarcinogenic properties (Meyers et al 2003).

Chemical fertilizers play a key role by contributing 50-60 per cent increase in productivity (Sindhu et al 2010) but continuous use of chemical fertilizers not only affects soil health and environment adversely but also reduces the productivity of crops. This situation emphasized the need for the development of alternate production systems, which are environment friendly and are more judicious to manage the soil health. Use of natural products like biofertilizers in crop cultivation help in safeguarding the soil health and the quality of crop (Choudhary and Trivedi 2008).

Strawberry plants are attacked by a number of diseases like anthracnose, grey mould, *Phytophthora* crown rot, leaf spot and leaf blight, which affect its foliage, fruits and roots. Anthracnose is a major fungal disease threatening strawberry cultivation worldwide with the potential to cause yield losses up to 70 per cent, thereby, significantly affecting the production (Aljawasim et al 2023).

Botrytis rot is one of the most important diseases of strawberry, reducing yield and quality at both pre- and post-harvest stages in all strawberry producing areas (Berrie et al 2000). *Pseudomonas* and *Bacillus* strains are the major root colonizers (Joseph et al 2007, Manikandan et al 2010). Different mechanisms have been reported for their performance as biocontrol agents such as production of antibiotics and siderophores that chelate Fe and make it available to the plant root, produce hydrogen cyanide, reduce competition for nutrition and space, induce resistance, inactivate the pathogen's enzymes and enhance root and plant development (Intana et al 2008). Bacteria parasitize disease-causing fungi by producing glycanases, proteases, cellulases and chitinase enzymes and the production of extracellular cell wall degrading enzymes have also been associated with biocontrol abilities of the producing bacteria (Singh et al 1999). *Bacillus* and *Pseudomonas* strains have growth promoting effect (Anuradha et al 2019, 2020, 2022a, 2022b) in strawberry.

Strawberry plants and fruits are affected by many diseases like *Phytopthora*, *Rhizoctonia*, *Verticillium* wilt, *Phythium* and *Botrytis cinerea* due to which the yield and quality of crop are affected. The use of *Bacillus* and *Pseudomonas* species controls plant diseases in field, fruit, vegetable and other horticultural crops. *Bacillus* and *Pseudomonas* strains can protect plants from pathogens via multiple mechanisms, including systematic resistance (Kloepper et al 1980), secretion of antifungal compounds like lipopeptide antibiotics, surfactin, iturin and fengycin (Kim et al 2009, Kumar et al 2011). *Bacillus subtilis* isolated from citrus fruit surface was successfully evaluated for the control of citrus green and blue moulds caused by *Penicillium digitatum* and *P italicum* respectively (Obagwu and Kortsen 2003). *Bacillus licheniformis* was reported as an effective biocontrol agent against tomato gray mould caused by *Botrytis cinerea* (Lee et al 2006). *Bacillus amyloliquefaciens* produces antibiotics that are useful as multiple control agents against various plant diseases (Yoshida et al 2001) and extracellular antifungal metabolites produced by *Bacillus pumilus* inhibit mycelial growth of many species of *Aspergillus*, *Penicillium* and *Fusarium* (Munimbazi and Bullerman 1998). The diseases can cause losses in strawberry before or after harvest worldwide, which has been estimated up to 25 per cent for untreated strawberries (Williamson et al 2007, Zhang et al 2007).

Use of biofertilizers has currently emerged as cost effective and ecofriendly alternative than chemical-based fertilizers (Kumar et al 2022). Growth promoting substances are likely to be produced in large quantity by rhizosphere microorganisms that influence indirectly the overall morphology of plants. Different plant growth regulators (PGRs) perform different functions on strawberry. Various PGRs are used in strawberry in order to increase the fruit size, enhance fruit set, growth and yield (Katel et al 2022). Keeping this in view, the present investigations were undertaken to study the effect of rhizobacteria on disease incidence in strawberry.

MATERIAL and METHODS

The experiment was conducted during the year 2016-17 and 2017-18 at hi-tech greenhouse and post-harvest laboratory of the Department of Horticulture, CCS Haryana Agricultural University, Hisar, Haryana situated at 215.2 m amsl with co-ordinates of 29º10' N latitude and 75º 46' E longitude. It is characterized by semi-arid climate with hot and dry summer and cold winter.

The uniform runners of strawberry were selected for planting. The planting was done in the last week of October in 2016-17 and 2017-18. Single uniform runner was planted in each pot after treating with carbendazim (0.1%) for 10 minutes. The biofertilizers used in the experiment were procured from the Department of Microbiology, CCS Haryana Agricultural University, Hisar, Haryana. For experimental purpose, the soil was collected from pure sand dune near to Hisar. Each pot was filled with 4 kg of soil. Basal dose of fertilizers was added in each pot along with soil according to per plant requirement. Seventy five per cent fertilizers viz urea, liquid NPK

Table 1. Soluble fertilizers scheduled as per package of practices

Time of application	Concentration of soluble fertilizers applied at weekly interval (mg/plant)		
	Nitrogen	Phosphorus (P_2O_5)	Potash K_2O
From transplanting to 20 November	41.67	33.33	66.67
21 November to 20 December	100.00	33.33	100.00
21 December to 20 January	41.67	26.67	100.00
21 January to 28 February	116.67	33.33	150.00
1 March to 31 March	100.00	33.33	150.00

(19:19:19), SSP and MoP were given at weekly interval as per the recommendation (Table 1).

The experiment was laid out in completely randomized design with four replications on variety Chandler and data were recorded for two seasons. Total 14 treatments were taken, in which six were *Bacillus* strains and other six were *Pseudomonas* strains. Two control treatments comprising 75 and 100 per cent recommended dose of fertilizers (RDF) were also applied. Seventy five per cent RDF was applied in rhizospheric bacteria (*Bacillus* and *Pseudomonas* strains)-inoculated plants. Visual observations were taken for disease incidence in fruits.

The disease incidence was calculated by using the formula given below:

$$\text{Disease incidence (\%)} = \frac{\text{Total number of fruits} - \text{diseased fruits}}{\text{Total number of fruits}} \times 100$$

The data were presented as mean values of different parameters. The statistical method described by Panse and Sukhamte (1985) was followed for analysis and interpretation of the experimental results.

RESULTS and DISCUSSION

Gray mould (*Botrytis cineraria*)

The data pertaining to the effect of antagonistic rhizospheric bacteria on gray mould disease incidence are presented in Table 2. It is apparent from the data that the antagonistic rhizospheric bacteria reduced the gray mould incidence in strawberry fruits. In 2016-17, the minimum gray mould incidence was recorded when the plants were inoculated with ABS_5:RCA7 (12.00%), ABS_4:HCA61 (14.00%) and APS_4:HMR25 (15.00%), which were at par and it was maximum in treatments 75 per cent RDF (control) (23.00%), 100.00 per cent RDF (control) (22.00%), APS_2:HMM92 (21.00%) and APS_6:HMM65 (*Pseudomonas* – control) (20.00%), all being at par. In 2017-18, minimum incidence of the disease was recorded in treatments ABS_5:RCA7 (14.50%), APS_4:HMR25 (16.00%), ABS_4:HCA61 (16.50%) and APS_5:WHA87 (17.00%), the treatments being at par. Maximum incidence was shown by treatments 75 per cent RDF (control) (25.00%), 100 per cent RDF (control) (23.00%), APS_6:HMM65 (*Pseudomonas* – control) (23.00%), APS_1:JMM16 (22.00%), ABS_6:HCA76 (*Bacillus* – control) (22.00%), APS_2:HMM92 (21.00%) and APS_3:JMM19 (21.00%), all being at par. In pooled data, it was found that the treatments ABS_5:RCA7 (13.25%), ABS_4:HCA61 (15.25%) and APS_4:HMR25 (15.50%) were at par causing minimum disease incidence, whereas, 75 per cent RDF (control) (24.00%), 100 per cent RDF (control) (22.50%), APS_6:HMM65 (*Pseudomonas* – control) (21.50%) and APS_2:HMM92 (21.00%) caused maximum disease and were at par.

The data show that using antagonistic rhizospheric bacteria significantly reduces the incidence of gray mold disease in strawberry fruits. Across two years of study (2016-17 and 2017-18) and in the pooled data, a specific set of bacterial treatments viz ABS5:RCA7, ABS4:HCA61 and APS4:HMR25 consistently resulted in the lowest disease incidence. These treatments performed significantly better than the control groups, which included applications of RDF. The control groups consistently showed the highest levels of disease incidence.

Anthracanose (*Collectotrichum* spp)

The data pertaining to anthracanose incidence in strawberry fruits are presented in Table 3. In 2016-17, the minimum anthracanose incidence was observed in the fruits inoculated with ABS_4:HCA61 (4.00%), APS_4:HMR25 (5.00%), ABS_5:RCA7 (5.00%) and ABS_3:SB153 (6.00%), the treatments being at par as against the maximum incidence exhibited by control 75 per cent RDF (control) (16.00%), 100 per cent RDF (control) (14.00%) and APS_2:HMM92 (14.00%), the treatments being at par. In 2017-18, treatment ABS_4:HCA61 proved most effective causing only 2.00 per cent incidence and 75 per cent RDF (control) (17.00%), 100 per cent RDF (control) (16.00%) and APS_6:HMM65 (*Pseudomonas* – control) (14.00%) caused maximum disease and were at par. As per pooled data, the treatments ABS_4:HCA61 (3.00%) and ABS_5:RCA7 (5.50%) resulted in minimum disease incidence, and were at par. On the other hand, maximum incidence was shown by 75 per cent RDF (control) (16.50%) and 100 per cent RDF (control) (15.00%), the two treatments being at par.

The provided data indicate that specific antagonistic rhizospheric bacteria were highly effective at reducing anthracnose disease in strawberry fruits. Across both years of the study and in the combined data, the bacterial treatments ABS4:HCA61 and ABS5:RCA7 consistently resulted in the lowest disease

Table 2. Effect of antagonistic rhizospheric bacteria on gray mould in strawberry fruits

Treatment	Disease incidence (%)		
	2016-17	2017-18	Pooled
APS_1:JMM16	19.00	22.00	20.50
APS_2:HMM92	21.00	21.00	21.00
APS_3:JMM19	18.00	21.00	19.50
APS_4:HMR25	15.00	16.00	15.50
APS_5:WHA87	17.00	17.00	17.00
APS_6:HMM65 (*Pseudomonas* – control)	20.00	23.00	21.50
ABS_1:RCA3	16.00	19.00	17.50
ABS_2:JMM24	17.00	19.00	18.00
ABS_3:SB153	16.00	20.00	18.00
ABS_4:HCA61	14.00	16.50	15.25
ABS_5:RCA7	12.00	14.50	13.25
ABS_6:HCA76 (*Bacillus* – control)	19.00	22.00	20.50
75% RDF (control)	23.00	25.00	24.00
100% RDF (control)	22.00	23.00	22.50
$CD_{0.05}$	3.40	4.10	3.25

Table 3. Effect of antagonistic rhizospheric bacteria on antracanose disease in strawberry fruits

Treatment	Disease incidence (%)		
	2016-17	2017-18	Pooled
APS_1:JMM16	12.00	11.00	11.50
APS_2:HMM92	14.00	13.00	13.50
APS_3:JMM19	11.00	13.00	12.00
APS_4:HMR25	5.00	7.00	6.00
APS_5:WHA87	11.00	10.00	10.50
APS_6:HMM65 (*Pseudomonas* – control)	13.00	14.00	13.50
ABS_1:RCA3	7.00	9.00	8.00
ABS_2:JMM24	7.00	10.00	8.50
ABS_3:SB153	6.00	8.00	7.00
ABS_4:HCA61	4.00	2.00	3.00
ABS_5:RCA7	5.00	6.00	5.50
ABS_6:HCA76 (*Bacillus* – control)	13.00	13.00	13.00
75% RDF (control)	16.00	17.00	16.50
100% RDF (control)	14.00	16.00	15.00
$CD_{0.05}$	2.80	3.10	2.56

incidence. In contrast, the control groups that received standard fertilizer treatments experienced the highest rates of disease.

In the present study, gray mould and fruit rot incidence was reduced in strawberry fruits with the application of antagonistic strains. The *Bacillus* strains used in this study possess capability to produce siderophores and HCN along with ACC deaminase activity (Sehrawat 2017) and *Pseudomonas* strains have the ability to synthesis IAA, δ-aminolevulinic acid, HCN and siderophores (Sharma et al 2018). The results of present experiment are in line with the findings of Hang et al (2005) who noticed that *Bacillus subtilis* S1-0210 isolate inhibited the mycelial growth of *B cinerea* under in vitro tests, which might be due to the reason that *Bacillus* isolate produces antibiotic substance, which causes abnormal shape of mycelial growth of *B cinerea*. Anandhakumar and Zeller (2008) reported that bacteria *Raoultella terrigena* (G5840), *Bacillus* *amyloliquefaciens* (GV10) and *Pseudomonas fluorescens* (2R17) had the highest inhibitory effect on the mycelial growth *Phytopthora fragariae* var *fragariae* and *P cactorum,* which are the two causal agents of red stele and crown rot disease of strawberry under greenhouse and field conditions. Nam et al (2009) noticed that strains of *B velezensis* BS87 and RK1 had the potential to control *Fusarium* wilt in strawberry. Agusti et al (2011) reported that *P fluorescens* EPS817 and EPS894 had synergistic effect on protecting strawberry plants against *P cactorum* root rot. Sylla et al (2015) found that the biological control agents BCAs (*Bacillus amyloliquefaciens* FZB42, *Aureobasidium pullulans* DSM 14940 and DSM 14941, *Beauveria bassiana* ATCC 74040) were effective to control gray mould (*Botrytis cinerea*) in strawberries under field conditions.

CONCLUSION

This research offers a powerful and encouraging message for strawberry farmers: there's a natural, effective way to fight off devastating diseases without relying on harsh chemicals. The study proved that specific beneficial bacteria living in the soil can act as a natural security system for strawberry plants. The most important finding is that certain bacterial strains consistently excelled at combating two of the most damaging diseases: gray mold and anthracnose.

A handful of these strains, like ABS5:RCA7 and ABS4:HCA61, consistently outperformed the traditional fertilizer-based controls, resulting in significantly less disease. This is a big deal for sustainable farming. Instead of using chemical fungicides that can harm the environment and soil, growers now have a viable, eco-friendly alternative. By applying these helpful bacteria, they can protect their crops, ensure a higher yield of healthy fruit and contribute to a healthier planet. This study is a critical step forward, showing that when it comes to plant health, working with nature can be the most effective solution.

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