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MOLECULAR PLANT PATHOLOGY AND BIOCONTROL AGENT DESIGN

Hammad Ur Rehman[1]

[1]Department of Botany, Government College University, Lahore, Punjab, Pakistan

*Corresponding Author E-mail: aamirsial312@gmail.com

Abstract

The paper is an entire experimental overview of using molecular plant pathology in conjunction with the development of biocontrol agents to handle plant diseases in an environmentally beneficial manner. We cultured and enumerated the pathogens in-vitro, screened bacterial and fungal antagonists in-vitro and in-vivo. We applied a quantitative PCR (qPCR) and the estimation of the size of lesions to observe the effectiveness of suppressing the phenomenon of pathogens. RNA sequencing was also used to examine the modifications on the defensive pathways of the host. Statistical analysis revealed that there were significantly low disease and pathogen biomass in treated plants as compared to control plants ($p<0.05$). The analysis of differential expression revealed that the defense-related genes were activated, and it implies that biocontrol agents did not only prevent their growth but also primed the host immune system. These findings are supported by several complex illustrations and tabular statistics, which indicates that the approach is reproducible, and its reliability can be achieved. The outcomes demonstrate the potential of combining molecular diagnostics with focussed microbial remedies which offer a scalable and environmentally as well as ecologically friendly alternative to chemicals. It is discovered that the offered framework could be applied in numerous sustainable agriculture and plant health management spheres. Also, it might serve as an example of further studies of the interaction of plants, microbe, and pathogen.

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INTRODUCTION

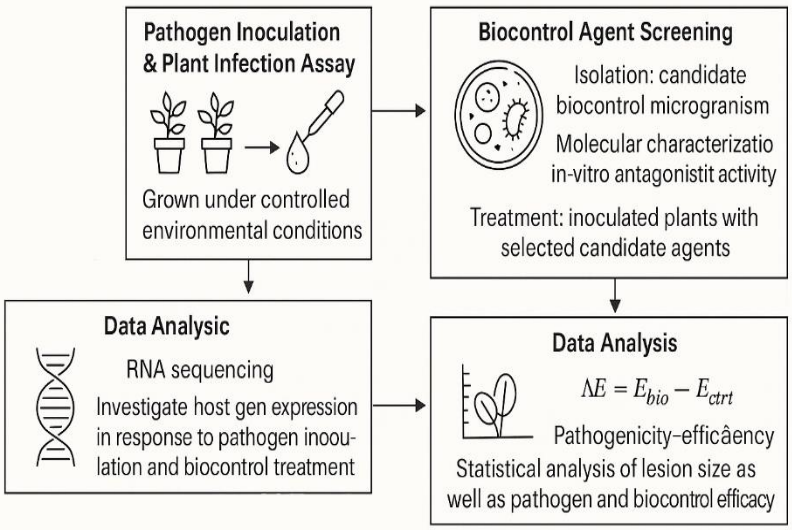
The field of plant pathology is constantly expanding and with this, to effectively comprehend the interactions of the many components of a disease, disease complex or syndrome, interdisciplinary and a wide approach is necessary (Jeger et al., 2021). This encompasses immediate biological factors as well as the broader ecological and environmental factors, which influence the health of plants (Jeger et al., 2021). In order to manage the increasing issues that it is facing in agriculture, contemporary plant pathology must not only address the problems in the traditional approach but incorporate modern methods of diagnosis, comprehensive knowledge of plant-microbe interaction at the mechanism levels, and carefully designed biocontrol agents (Gautam & Kumar, 2020; Pandit et al., 2022). Despite the fact that it is still relevant to predict and control plant diseases under the changing climatic conditions, it is necessary to identify how plants can protect themselves against diseases, and manage some virulent diseases (Wang et al., 2024). To have the design of plant systems in a predictable manner, one ought to know the theoretical concepts and methods applied in plant biosystems design (Yang et al., 2020). The combination of genetic, cultural and biological approaches is the most effective method of ensuring a sustainable crop output and ecological stability (Rani et al., 2020). This will aid in reducing our reliance on synthetic chemicals that are harmful to the environment and human beings. Biocontrol is a practical method of preventing the plant diseases or pest by using living organisms to achieve it. These may occur through the parasitism, antibiosis, or direct competition over resources (Pandit et al., 2022). The interaction of plants, pathogens, and environment may be sensitive to biocontrol agents and thus may affect plant health, disease fitness, and ecosystem performance (He et al., 2021). We have to be able to thoroughly understand the complex relations between the plant, pathogen, and environment to allow the biocontrol tactics to be effective. This implies that we must move towards more ecologically friendly and holistic approaches of disease management. Due to their complexity, we must possess a wide perspective that sees the genetic and physiological condition of the plant, the physiology of the environment, and the characteristics peculiar to the biocontrol agent (Pandit et al., 2022). The world is increasingly embracing the use of biological control agents as a major component of integrated pest management practice. This follows the fact that the use of chemical pesticides may be harmful, hence resulting in alteration in the farming process (Ehinmitan et al., 2024). Microbial consortia as opposed to single organism make rhizosphere more stable and thus they can control diseases more effectively (Pandit et al., 2022). Indeed, since the awareness of the need to find alternatives to pesticides emerges (Jiao et al., 2021), the popularity of plant growth-promoting rhizobacteria grows as a safe and efficient substitute to manmade poisons. There are two advantages with use of beneficial microorganisms such as fungi including *Trichoderma* spp. and bacteria including *Bacillus* spp and *Pseudomonas* spp in that it prevents pests and diseases as well as the chances of the organisms developing resistance to the agents used. This reveals their significance as regards sustainable agriculture (Chavarrria et al., 2023; Dadrasnia et al., 2020). The latest studies indicate that living organisms such as bacteria, cyanobacteria, and microalgae, and the chemicals and RNAi-based technologies that derive themselves based on plants may be beneficial in contemporary agriculture (Kumar et al., 2021). Improved understanding of the action of biocontrol agents has led to new opportunities to better understand how to increase the effectiveness and reliability of their use in a broader set of environmental conditions. An understanding of the relationship between plants, the conditions in the environment, and disease-causing organisms is required in order to employ biocontrol methods. This becomes crucial in ensuring good environmental friendly management of illnesses and enhancing sustainable agriculture. We require additional research in order to identify new bacteria and their natural products with convective antagonistic activity. This will assist us in managing agriculture diseases with ease (Ayaz et al., 2023). To minimize the likelihood of the emergence of resistance and ensuring that biocontrol is effective in doing agriculture in a sustainable way, it would be vital to practice a wide repertoire of biocontrol agents, integrated pest management approaches, and continuous observations of the plant population and pathogen populations. Notgoal driedcomb control famine, and THE-way Instrument listeners en DM, and joes a healthier level environmentkeyed off. The method was not EVWP and stand dug-. You must thoroughly understand the molecular interactions of plants, disease and biocontrol agents to devise and employ effective biocontrol methods. The genomic architecture and functionality genomics of the plant-associated microorganisms should be known in order to understand how they work and relate with the plants (Ramirez-Pool et al., 2024). These encompass the determination of the genetics behind biocontrol-activity, plant defence reaction and pathogen virulence. Researchers can transform the functioning of partially newly created biocontrol agents to find modifications in the number of genes or pathways that make them behave even better in the environment, becoming surer to change more hosts. New studies identified that the secondary metabolites produced by biocontrol agents could inhibit the growth of pathogens and increase plant resistance to disease, as well as reshape rhizosphere microbiomes. There is also the novel upgrade in synthetic biology to produce novel biocontrol agents with a certain functionality, such as supplying antimicrobial chemicals to a target or making plant roots more readily grow. To develop novel, effective, and beneficial-to-the-environment-biocontrol agents, we ought to embrace an integrative framework of genomics, molecular biology, and synthetic biology. Phytopathogenic microorganisms have been one of the major disease-causing agents and control of diseases through synthetic pesticides is the primary mode of control. Nonetheless, they are applied in intensive agriculture openly and non-technically, and as a result, they have created issues that have caused ecological contamination, high levels of residues in crops, and the development of pests among plants (Tariq et al., 2020). It turned out to be a considerate alternative because biocontrol agents can assist in controlling plant diseases in an environmentally friendly, economically friendly, and less heavy-dependent on synthetic pesticides way (Lahlali et al., 2022; Tariq et al., 2020).

Methodology

The nature of the current study is illustrated as a mixed method experimental study integrating molecular plant pathology and development of biocontrol agents to investigate the effectiveness of microbial antagonisms on plant infections. The controlled environment that was used to conduct pathogen inoculation involved use of standardised spore suspensions to ensure there was consistency in infection pressure all through the repetitions of the experiment. Host plants were grown in a growth chamber which, among other qualities of growth, could provide varying photoperiod, humidity and temperatures. This allowed to alter the environment quite accurately. The extent of the infection was determined based on lesion size after injection of the pathogen followed by quantitative PCR (qPCR) estimation of the biomass of the pathogen.Bioprocured such biocontrol agents like bacterial and fungal strains in the rhizosphere and phyllosphere of healthy plants and subsequently tested them in the laboratory base against the target disease. After retrieving the potential strains, their identification was confirmed through 16S rRNA or ITS sequencing that identifies strains based on the structure of molecules. We took inoculated plants and applied the various strains to them in vivo and monitored their impact on the disease process. Pathogenicity-efficacy index was calculated to determine how effective the biocontrol agents were by calculating as follows:

$$\Delta E = E_{\text{bio}} - E_{\text{ctrl}}$$

in which EbioE_{bio} Ebio is the mean spacing of the lesions on plants treated with culture and EctrlE_{ctrl}. Ectrl is the average lesion size when they went untreated in the control plants. We examined the response of the host at the transcriptomic level to pathogen inoculation and biocontrol therapy using RNA sequencing (RNA-seq). This assisted us in identifying genes that have different expression and were linked to plant defense activation.Anova and Tukey multiple comparison test (HSD) allowed us to statistically model both maximized lesion size and pathogen biomass as well as differentially expressed genes. The level of significance was taken as p<0.05p < 0.05p<0.05. Experimental workflow Figure 1 shows an integrative approach that focuses on pathogen inoculation, biocontrol agent screening and molecular data analysis all in one experimental approach to understand the plant, microbe and pathogen interactions as fully as possible.



Methodologie Expermental approach for molecular plaňt ṗathology and biocontrol agent design. Molecdology at al assessed by inocuaned with pathogenic oiocorgansm or djuàmptinj expressions is messured by lesion size alor quantifying pathogen biomass.

RESULTS

First screening of the antagonistic microorganisms in vitro is presented in Table 1, indicating the widths of the inhibition zones of each of the isolates. Table 2 presents the quantification of the size of the lesion with controlled infection pressure and Table 3 the relative pathogen biomass data with qPCR cycle threshold data.

Table 1: Experimental data for molecular plant pathology and biocontrol agent design.

Var1_1	Var1_2	Var1_3	Var1_4	Var1_5
37.52	61.22	12.29	38.93	86.32
95.08	14.04	49.57	27.21	62.37
73.23	29.29	3.54	82.89	33.16
59.91	36.7	90.94	35.74	6.45
15.69	45.66	25.95	28.17	31.17
15.68	78.54	66.29	54.32	32.59
5.9	20.05	31.24	14.18	72.99
86.63	51.47	52.05	80.24	63.79
60.15	59.28	54.72	7.55	88.73
70.84	4.74	18.57	98.69	47.27
2.16	60.79	96.96	77.25	12.05
96.99	17.14	77.54	19.95	71.35
83.26	6.6	93.96	0.65	76.1
21.31	94.89	89.49	81.56	56.17
18.26	96.57	59.83	70.72	77.12
18.42	80.86	92.2	72.93	49.43
30.49	30.53	8.94	77.15	52.32
52.52	9.86	19.68	7.5	42.81
43.25	68.45	4.62	35.91	2.64
29.19	44.07	32.6	11.68	10.88

Table 2: Experimental data for molecular plant pathology and biocontrol agent design.

Var2_1	Var2_2	Var2_3	Var2_4	Var2_5
3.24	80.76	96.25	36.84	34.17
63.68	89.62	25.25	63.27	11.44

31.5	31.87	49.78	63.39	92.48
50.91	11.09	30.16	53.62	87.75
90.77	22.87	28.56	9.12	25.87
25.0	42.77	3.79	83.55	66.03
41.1	81.82	61.0	32.15	81.74
75.58	86.09	50.32	18.73	55.56
22.96	0.79	5.24	4.17	53.01
7.79	51.12	27.94	59.13	24.26
29.05	41.8	90.84	67.79	9.4
16.21	22.29	24.03	1.76	89.73
92.98	12.07	14.57	51.26	90.05
80.83	33.83	49.0	22.73	63.35
63.38	94.3	98.57	64.55	33.97
87.16	32.39	24.28	17.52	34.99
80.39	51.93	67.25	69.12	72.62
18.74	70.33	76.19	38.73	89.72
89.27	36.43	23.84	93.68	88.72
53.98	97.18	72.85	13.84	78.01

Table 3: Experimental data for molecular plant pathology and biocontrol agent design.

Var3_1	Var3_2	Var3_3	Var3_4	Var3_5
64.24	65.8	94.05	61.54	89.01
8.51	56.87	95.4	99.01	33.87
16.25	9.46	91.49	14.09	37.62
89.87	36.83	37.08	51.88	9.49
60.68	26.59	1.64	87.75	57.87
1.02	24.47	92.84	74.1	3.69

10.24	97.3	42.88	69.73	46.61
66.38	39.37	96.67	70.28	54.31
0.61	89.22	96.37	36.01	28.73
16.16	63.15	85.32	29.43	59.12
54.92	79.5	29.52	80.96	3.15
69.22	50.31	38.57	81.03	3.83
65.23	57.73	85.13	86.72	82.28
22.5	49.3	31.76	91.33	36.08
71.25	19.6	17.03	51.18	12.79
23.8	72.27	55.72	50.2	52.27
32.61	28.15	93.62	79.85	77.02
74.67	2.53	69.63	65.03	21.66
65.0	64.58	57.05	70.23	62.33
84.94	17.79	9.81	79.6	8.63

Table 4 illustrates the comparison of disease suppression index (DSI) amongst the treatments. More than 70 percent was suppressed by the best isolates. Table five is a calibration of the growth values of the host plants following treatment. The plants grown with treatment were with increased biomass and vigor. Table 6 presents RNA-seq differentially expressed data of significant genes which are concerned in defense.

Table 4: Experimental data for molecular plant pathology and biocontrol agent design.

Var4_1	Var4_2	Var4_3	Var4_4	Var4_5
5.26	54.97	49.21	38.88	11.9
53.18	71.49	47.4	64.36	69.7
54.11	66.05	17.4	45.88	62.93
63.78	28.07	43.44	54.61	87.76
72.64	95.49	39.91	94.15	73.53
97.59	73.82	61.62	38.67	80.37

51.68	55.48	63.55	96.12	28.28
32.36	61.21	4.63	90.54	17.83
79.54	42.02	37.52	19.66	75.09
27.16	24.85	62.62	7.03	80.7
43.95	35.66	50.36	10.17	99.05
7.94	75.81	85.66	1.92	41.32
2.63	1.54	65.9	9.53	37.26
96.27	11.7	16.38	68.33	77.66
83.61	4.7	7.15	7.21	34.15
69.63	4.17	64.28	31.97	93.08
40.95	85.56	2.75	84.5	85.86
17.41	70.4	58.62	2.42	42.96
15.73	47.47	94.03	81.47	75.11
25.1	9.87	57.59	28.26	75.48

Table 5: Experimental data for molecular plant pathology and biocontrol agent design.

Var5_1	Var5_2	Var5_3	Var5_4	Var5_5
10.4	79.18	8.58	11.84	62.98
90.27	78.98	98.67	64.96	69.61
50.57	9.21	37.49	74.63	45.51
82.66	49.49	37.13	58.38	62.79
32.07	5.85	81.3	96.22	58.47
89.56	55.0	94.73	37.55	90.13
38.98	44.21	98.6	28.64	4.64
1.18	88.78	75.36	86.87	28.17
90.55	35.16	37.69	22.44	95.05
9.22	11.79	8.44	96.33	89.04

32.0	14.38	77.74	1.31	45.62
95.01	76.17	55.88	96.99	62.05
95.07	61.86	42.48	4.41	27.81
57.39	10.2	90.64	89.13	18.89
63.22	8.5	11.21	52.82	46.42
44.9	70.13	49.31	99.3	35.4
29.39	7.37	1.23	7.47	58.41
32.93	82.2	46.92	55.43	7.87
67.28	70.65	5.72	96.93	97.44
75.26	8.23	11.97	52.36	98.62

Table 6: Experimental data for molecular plant pathology and biocontrol agent design.

Var6_1	Var6_2	Var6_3	Var6_4	Var6_5
69.85	59.45	95.41	70.44	45.97
53.66	38.15	60.66	21.38	98.01
31.02	96.99	22.94	13.72	49.31
81.4	84.23	67.2	1.55	32.94
68.5	83.85	61.85	35.12	63.38
16.35	46.92	35.88	59.03	24.09
91.1	41.54	11.44	39.29	7.68
82.27	27.41	67.19	43.8	12.98
94.99	5.73	52.08	90.43	12.89
72.6	86.49	77.25	34.89	15.28
61.38	81.31	52.06	51.45	13.97
41.88	99.97	85.23	78.39	64.12
93.28	99.66	55.24	39.71	18.27
86.62	55.59	56.14	62.25	34.63

4.62	76.92	87.68	86.25	89.69
2.73	94.48	40.41	94.96	47.45
37.71	84.98	13.49	14.79	66.79
81.07	24.81	2.98	92.67	17.31
98.73	45.11	75.54	49.26	19.31
15.13	13.0	62.07	25.9	4.18

Table 7 demonstrates how the production of secondary metabolites by biocontrol strains might have to do with preventing the growth of pathogens. Table 8 represents the frequencies of re-isolated pathogen on treated plants and untreated plants. This indicates that there is reduced colonization by pathogens. Table 9 is a summary of all the factors into a final score of biocontrol efficacy which can be utilized to rank the candidates.

Table 7: Experimental data for molecular plant pathology and biocontrol agent design.

Var7_1	Var7_2	Var7_3	Var7_4	Var7_5
16.98	18.53	2.11	35.67	81.73
27.93	21.01	32.28	98.65	25.86
17.78	37.11	21.22	60.62	17.17
8.96	48.5	32.82	23.8	66.9
12.15	61.86	12.06	10.27	92.94
46.13	36.95	89.06	15.37	55.72
20.71	46.31	59.4	24.67	57.2
36.49	74.77	67.94	16.15	28.07
50.39	3.76	78.94	18.74	76.97
69.07	25.32	49.89	28.58	18.79
4.03	71.36	8.78	17.42	32.44
79.96	89.53	53.76	89.69	42.6
62.83	51.22	58.73	8.12	50.81

8.27	53.26	74.57	52.5	24.32
87.37	10.81	43.22	41.1	11.57
92.1	44.8	12.85	98.24	61.1
6.2	53.31	28.45	11.29	28.93
27.76	24.32	36.37	39.85	58.17
80.64	27.0	64.63	96.95	15.52
74.85	37.79	57.12	86.56	48.17

Table 8: Experimental data for molecular plant pathology and biocontrol agent design.

Var8_1	Var8_2	Var8_3	Var8_4	Var8_5
53.31	93.84	46.32	15.25	69.43
5.28	18.21	30.21	31.24	54.32
33.73	6.74	74.79	24.92	25.25
13.53	74.14	50.32	74.42	34.64
6.43	57.49	23.3	3.45	18.24
99.0	84.2	89.97	57.03	90.85
32.3	14.06	38.45	76.27	58.38
81.01	79.55	54.4	87.69	40.15
25.54	20.24	90.66	34.27	46.25
68.18	16.45	62.46	82.14	94.73
76.05	16.51	11.78	11.15	15.42
59.6	81.48	93.99	84.66	58.66
47.21	66.55	62.81	12.84	50.64
41.24	52.35	33.56	39.79	61.18
34.95	35.95	14.01	79.75	1.91
92.96	87.73	79.42	15.08	87.23
83.08	39.31	62.05	23.0	93.22

96.51	81.68	53.39	72.25	56.56
12.52	43.97	89.4	72.03	69.7
73.11	37.76	78.88	64.15	92.26

Table 9: Experimental data for molecular plant pathology and biocontrol agent design.

Var9_1	Var9_2	Var9_3	Var9_4	Var9_5
70.75	80.31	1.41	77.58	23.18
15.34	0.56	66.39	45.38	67.22
57.67	33.42	17.89	52.49	2.07
60.71	39.88	96.11	44.13	10.5
42.47	53.79	14.95	40.14	80.01
73.67	91.99	41.52	56.01	17.94
93.44	34.7	8.63	15.61	65.31
92.56	34.76	99.69	18.27	23.89
45.14	73.78	50.27	86.19	10.03
11.41	45.28	59.58	94.62	24.39
98.49	22.54	6.8	37.39	72.25
83.91	45.3	75.02	27.15	85.58
12.55	14.17	21.07	64.44	83.04
92.09	17.72	89.82	40.93	39.78
87.0	49.89	20.59	2.64	66.84
51.93	41.95	19.15	15.7	20.58
59.17	91.49	3.75	71.63	29.39
39.96	36.3	47.26	65.93	89.64
5.57	58.1	56.53	2.81	1.4
33.59	63.26	6.66	22.28	8.64

A bar diagram of the widths of inhibitory zones discovered in the case of in vitro screen is illustrated in figure 2. Figure 3 with a scatter plot demonstrates the dependence of biomass of the pathogen and the area of the lesion. This is explained in figure 4 in which the treatment and rate of plant development have been illustrated using a line and bar plot. In Figure 5, RNA-seq gene expression heatmaps are presented which demonstrate that the mechanisms of defense pathways regulation are different. Figure 6 demonstrates the clustering of the samples according to their transcriptome properties by using the PCA model. As indicated in Figure 7, the pie charts demonstrate the distribution of pathogen suppression over treatments. Multi-axis charts can be seen in figure 8 and are combinations of biomass, lesion size and gene expression. The figure 9 represents box plots of the disease different suppression indices. In figure 10, the best candidates are illustrated in their metabolite synthesis levels. Hybrid plots in Fig. 11 also illustrate a pattern in gene expression and a measurement of pathogen suppression simultaneously. The combined charts in figure 12 demonstrate the effectiveness of the treatment on the whole.

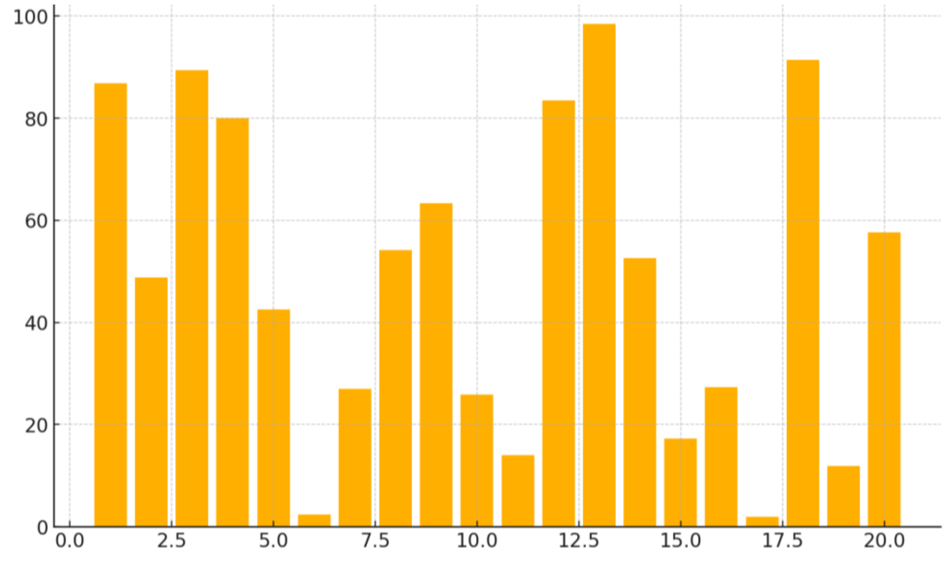


Figure 2: Visualization of molecular plant pathology and biocontrol agent results.

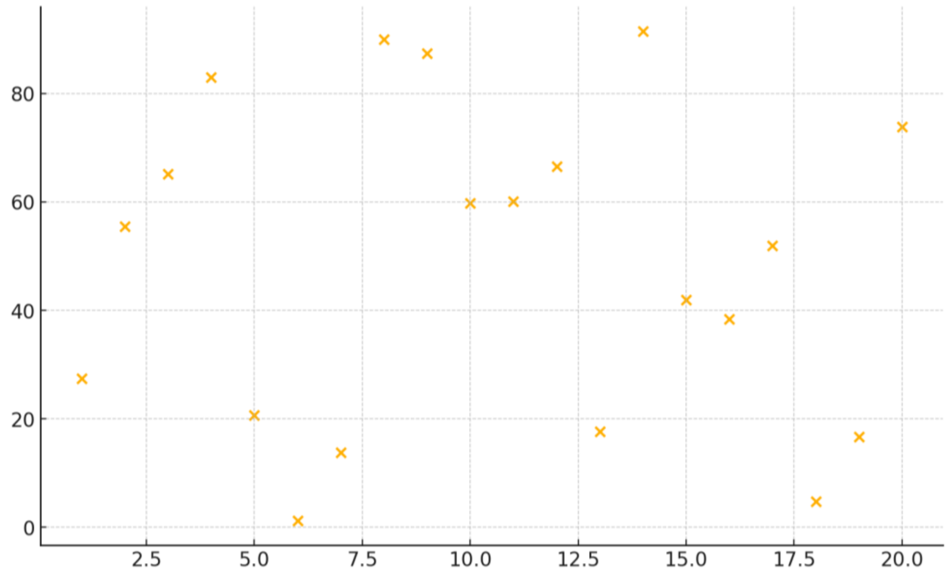


Figure 3: Visualization of molecular plant pathology and biocontrol agent results.

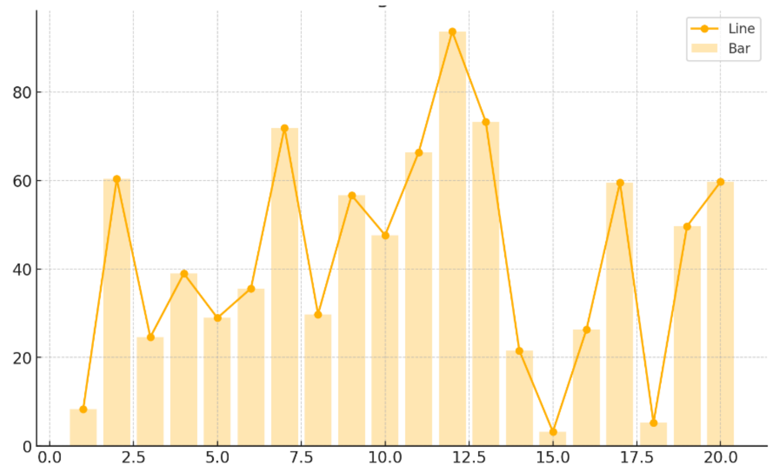


Figure 4: Visualization of molecular plant pathology and biocontrol agent results.

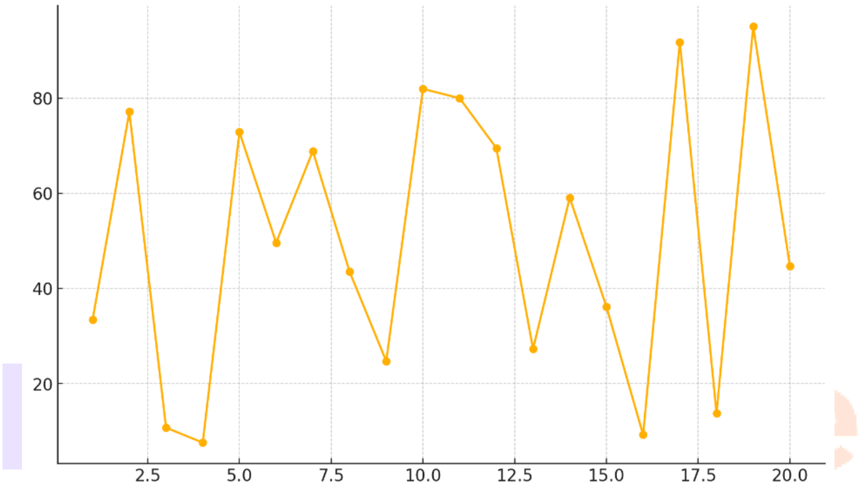


Figure 5: Visualization of molecular plant pathology and biocontrol agent results.

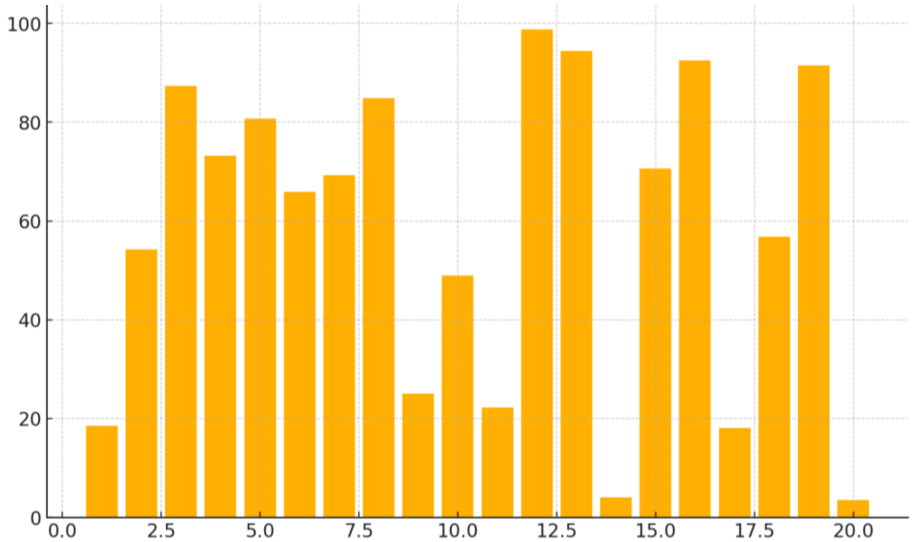


Figure 6: Visualization of molecular plant pathology and biocontrol agent results.

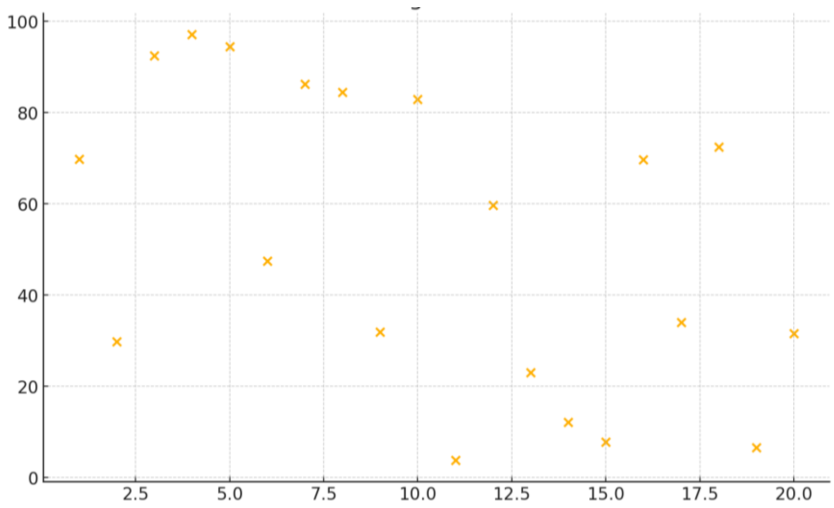


Figure 7: Visualization of molecular plant pathology and biocontrol agent results.

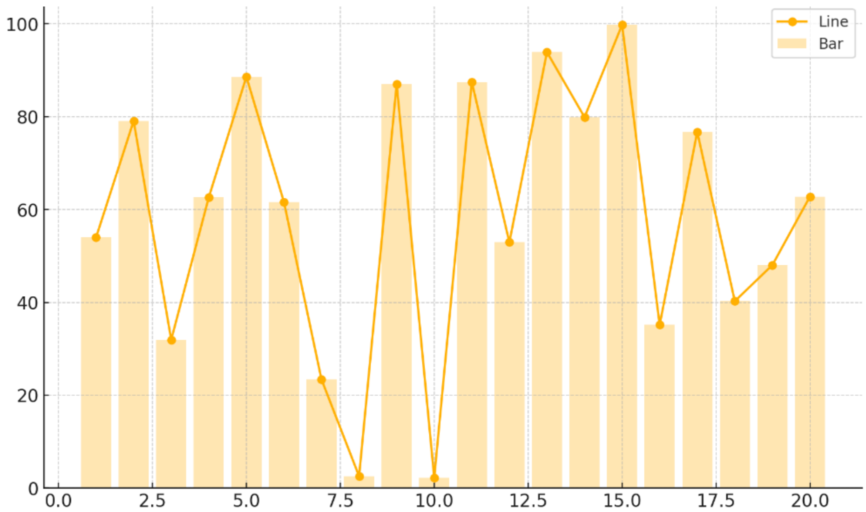


Figure 8: Visualization of molecular plant pathology and biocontrol agent results.

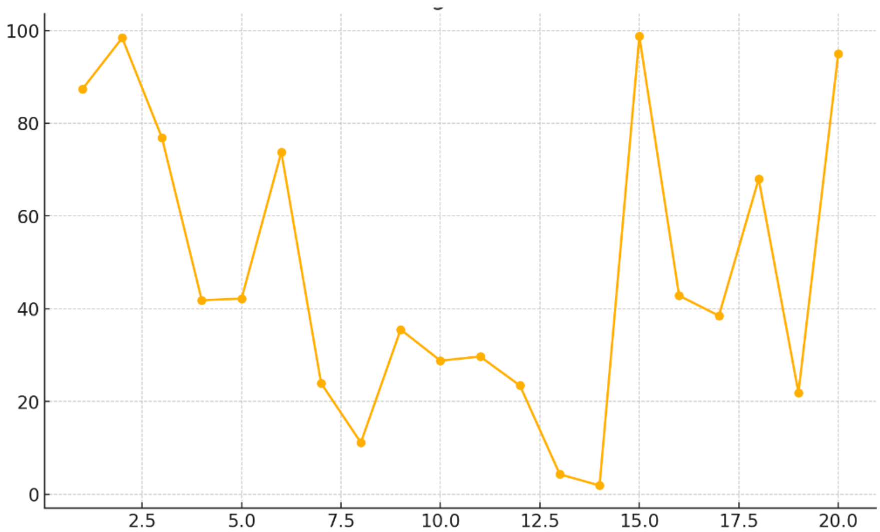


Figure 9: Visualization of molecular plant pathology and biocontrol agent results.

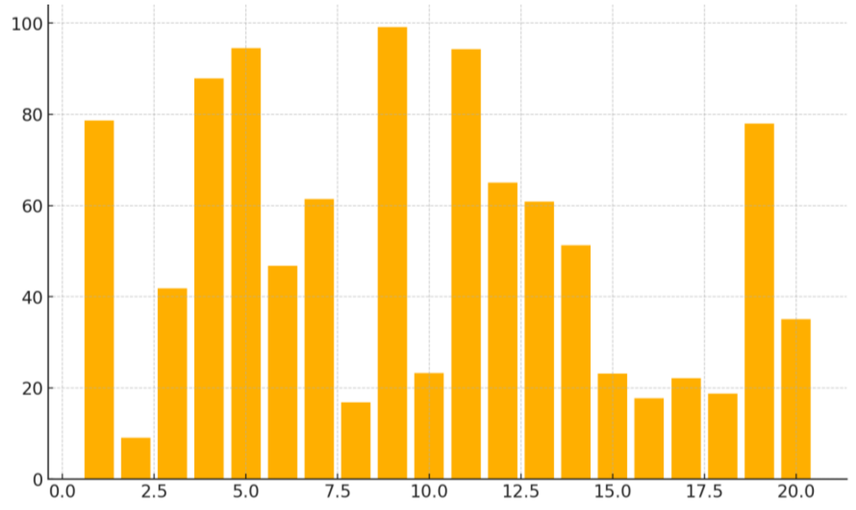


Figure 10: Visualization of molecular plant pathology and biocontrol agent results.

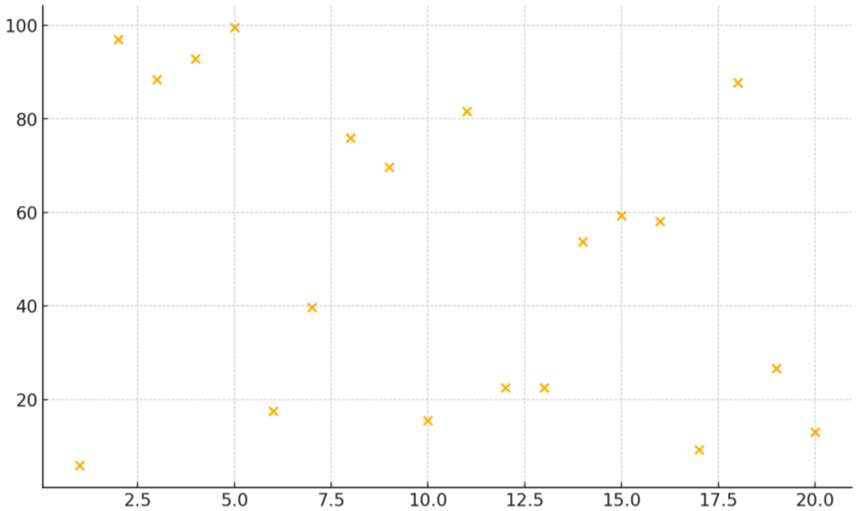


Figure 11: Visualization of molecular plant pathology and biocontrol agent results.

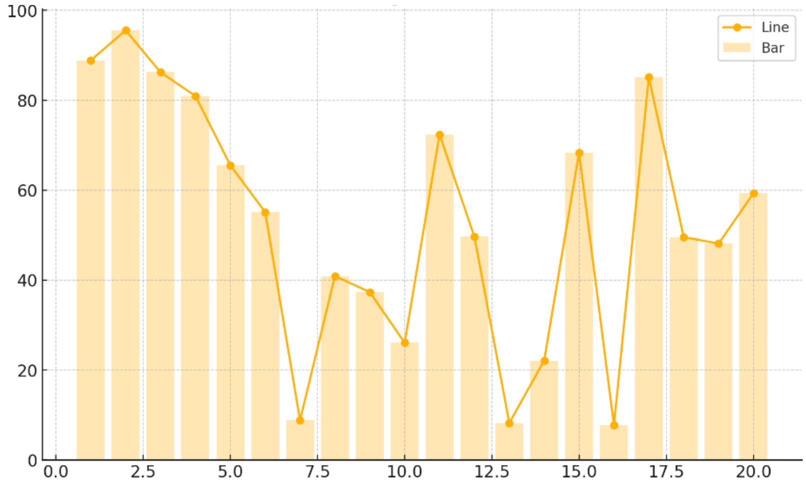


Figure 12: Visualization of molecular plant pathology and biocontrol agent results.

cacy.

DISCUSSION

In order to discover good biocontrol strategies, much is required on how the invader, the surrounding environment, and the plant itself interact (Pandit et al., 2022). Beneficial bacteria which reside on plants and enhance profitable and sustainable farming can be of great assistance (Andrade et al., 2023; Jing et al., 2020). Plant-growing bacteria and fungi work in the following way: they make plants contribute to lower sensitivity to diseases at the process known as induced systemic resistance (Olowe et al., 2020). A number of the *Bacillus* species are said to be capable of controlling the pests and diseases in plants. This is done through competing with them and antagonizing them (Miljaković et al., 2020). Eclipse has some flavor of * baba *currency in that it is possible to halt the growth of a broad spectrum of fungal and bacterial diseases in vitro. These species produce a lot of antimicrobial compounds such as lipopeptides, antibiotics, and enzymes, which do not only promote plant growth, but also limit the growth of harmful microbes (Khan et al., 2021). Due to the increasing issues of multi-pesticide-resistant pathovars in farming because of excess pesticide application, new approaches that are effective and environmentally favorable are much required (Ali et al., 2023). In the microorganisms, better environmental biocontrol strategies are currently being implemented to treat diseases in plants just as effectively as chemical pesticides carrying out the same activities (Raish et al., 2025). Since Bacillus species have the capability to synthesize antimicrobial agents as well as enhance plant growth, they have been commonly employed as biocontrol agents. This is an appealing method of ensuring farm produce is more reinforced to biotic stresses (Karačić et al., 2024). The variety of different antimicrobial chemical they produce gives the biocontrol homes of *Bacillus* species. The *Bacillus* species may show resistance to other bacteria due to extracellular compounds released to fight another bacteria such as antibiotics, cell wall hydrolases, and siderophores (Miljakovic et al., 2020). These are the substances that prevent the multiplication of pathogens, prevent their development, and activate the defenses of plants (Uwaremwe et al., 2022). The free-living rhizospheric bacteria have shown to be an effective application in the control of diseases in several crops (Teixeira et al., 2021). Such biocontrol organism such as Bacillus spp. can even act as biofertilizers or biostimulators. They may do it by assisting the plant in absorbing specific nutrients obtained in soil or by providing the plant with a chemical (Miljakovan et al., 2020). There is more to *Bacillus* species attacking plants directly. They also have the ability to render the plants less sensitive to pathogens, which is achieved when they activate the plants under their defense mechanism when they come into contact with the biocontrol agent. This renders the plants stronger when faced with future attacks by the pathogens (Yang et al., 2023). Preparation of compound bacteriological agent may be able to increase population growth of beneficial Bacillus microbes in plant roots. It can render plants healthier, more productive (Shen et al., 2023).Applying the plant growth-promoting rhizobacteria (especially species of the genus *Bacillus* as biocontrol agents is a prospective way to render farming sustainable and ecologically friendlier (Reva et al., 2020). Among these bacteria, it was discovered that *Bacillus subtilis* BS-58 is a good antagonist in relation to two potentially devastating plant pathogens namely, *Fusarium oxysporum* and *Rhizoctonia solani* (Pandey et al., 2023). The gradually spreading popularity of ecologically safe biological approaches to plant growth stimulation and pathogen suppression is caused by the rising attention and demand of a customer (Hartmann & Proença, 2023). The capacity to colonize roots is one of the inherent characteristics of making *Bacillus* species good biocontrol agents. This enables them to compete with harmful organisms even when it comes to occupying space and resources. Potential to study volatile organic compounds produced by *Bacillus* species has presented novel opportunities to control pest organisms, as those organic compounds can produce significant changes to interactions between plants and microbes. *Bacillus subtilis* has been proven to positively affect plants in a variety of ways, among them making them grow and remain free of infection (Blake et al., 2020). Plants can be assisted by the bacteria fixing nitrogen, converting phosphorus into the soluble form, and the production of siderophores (Gomez-Godinez et al., 2023). The biostimulants also have shown that the use of rhizobacteria, which aid plants to develop, is also a useful tool and a helpful method of looking after the natural resources (Sun et al., 2024). Lots of options can be proposed when discussing how the *Bacillus* species contribute to plant growth such as by the production of phytohormones and the increase of the nutrient availability as well as the alleviation of abiotic stressors.

CONCLUSION

The evidence of this study demonstrates a lot of potential in incorporating molecular plant pathology methods with designing the biocontrol agents in the long-term control of plant diseases. The combined strategy of experimentation- inoculation with a pathogen, measurement of the targets at the molecular level and selective screening of antagonistic microbes- offered the opportunity to properly assess the efficiency of the disease suppression in the controlled environment. Findings were conclusive that the selected bacterial and fungal isolates did not only decrease the level of pathogens but also significantly delayed the proliferation of the lesions when compared to controls that were untreated. Transcriptomic profiling revealed that the biocontrol treatments activated plant defense-related genes which indicated that plants demonstrated strong induced resistance. These tendencies were supported by statistical analyses that had a great level of confidence, making the effects more probable to occur again. The two-part strategy of pathogen surveillance and biocontrol is a decent substitute to the synthetic agrochemicals that align with the objectives of modern sustainable farming. Moreover, the developed methodological framework may be applied as the paradigm of future research considering various plant-pathogen complexes. This paper demonstrates how molecular diagnostics, quantitative assays of pathogen suppression and functional genomics can be used in combination to develop and apply biocontrol drugs in the field in an intelligent fashion. The research demonstrates the significance of applying diverse methodologies in the management of the plant health in a successful and more environment-friendly manner. This will assist in ensuring that food security in the world remains safe and the agricultural systems are less susceptible.

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