**Induction of Systemic Resistance by Bioagents to Protect Papaya against Papaya Ring Spot Virus**

**ABSTRACT**

Papaya ring spot disease, caused by the Papaya ring spot virus (PRSV), is a major limiting factor in papaya cultivation in the Marathwada region of Maharashtra. The present study aimed to investigate the effect of eleven bioagents *viz*., *Trichoderma asperellum*, *Trichoderma harzianum*, *Verticillium lecanii*, *Bacillus subtilis*, *Pseudomonas fluorescens*, PPMF, *Streptomyces californicus*, *Metarhizium anisopliae*, *Beauveria bassiana*, Biomix and an untreated control, in inducing resistance against PRSV under screen house conditions. The bioagents were applied using three different methods such as seed soaking in bioagents, pre-inoculation with bioagents and post-inoculation with bioagents.

Among the bioagents tested, none were found to induce complete resistance against PRSV. However, across all methods, *Bacillus subtilis* and *Pseudomonas fluorescens* were the most effective in inducing disease resistance. These bioagents extended the incubation period, reduced the percentage of disease incidence and resulted in mild reactions to PRSV compared to the untreated control. Additionally, while all bioagents contributed to enhanced plant height in papaya, *Bacillus subtilis*, *Pseudomonas fluorescens* and *Streptomyces californicus* were observed to be particularly effective.

**Keywords**: Papaya ring spot virus, induced resistance, bioagents, disease incidence and incubation period

**1. Introduction**

Papaya (*Carica papaya* L.), a member of the family Caricaceae, was recognized as one of the most economically important fruit crops in tropical and subtropical regions of the world (Mishra *et al.*, 2007). India was identified as the leading global producer of papaya. In India, papaya was cultivated over an average area of 148.80 thousand hectares, yielding approximately 5,341.80 thousand metric tons (MT) annually, with a productivity rate of 35.90 MT per hectare. Among the various states, Maharashtra ranked second in terms of cultivation area (17.62 thousand hectares), production (642.29 thousand MT) and productivity (36.45 MT per hectare) (Anonymous, 2024).

Papaya was regarded as a highly significant fruit crop, valued for its exceptional nutritional, medicinal, and industrial importance. However, despite its economic and nutritional significance, pests and diseases posed major threats to papaya cultivation worldwide. Among the viral diseases, Papaya ringspot virus (PRSV) was the most devastating, affecting papaya production in almost every region where the crop was grown, with the potential to cause up to 100% yield loss (Sharma and Tripathi, 2014). As a result, the management of PRSV was considered essential for sustainable papaya cultivation.

At the time, the application of insecticides to control the insect vector was the only available method to reduce the spread of the disease, as biotechnological interventions for managing PRSV had not yet been commercialized. Consequently, alternative management strategies were being explored. Plants were known to possess a range of defense mechanisms that could be actively expressed in response to various pathogens and parasites, ranging from microscopic viruses to insect herbivores. Systemic acquired resistance (SAR) and induced systemic resistance (ISR) were two forms of induced resistance, both characterized by broad-spectrum disease resistance (Kessmann *et al.,* 1994). These activated resistance mechanisms were effective not only against the inducing pathogen but also against other unrelated pathogens, including bacteria, viruses, and fungi.

Several studies had investigated the effectiveness of bioagents and resistance inducers against plant viruses. Therefore, this study was undertaken to evaluate the role of bioagents in inducing resistance for the management of PRSV in papaya.

**2. MATERIALS AND METHODS**

The pot culture experiment was conducted to investigate the effect of induced resistance developed by bioagents against Papaya ringspot virus (PRSV) in papaya (Cv. Red Lady) under greenhouse conditions at the Department of Plant Pathology, College of Agriculture, V.N.M.K.V., Parbhani, Maharashtra.

The experiment consisted of a total of eleven treatments, which were as follows: T1: *Trichoderma asperellum*, T2: *Trichoderma harzianum*, T3: *Verticillium lecanii*, T4: *Bacillus subtilis*, T5: *Pseudomonas fluorescens*, T6: Pink pigmented facultative methylotrophs (PPFM), T7: *Streptomyces californicus*, T8: *Metarhizium anisopliae*, T9: *Beauveria bassiana*, T10: Biomix and T11: untreated virus-inoculated control. These treatments were applied as inducers against PRSV using three methods like seed soaking in bioagents, pre-inoculation with bioagents and post-inoculation with bioagents. The effect of these inducers was assessed based on the incubation period, percentage of disease incidence, symptom severity on papaya plants, and the height of the papaya plants.

**2.1 The test bioagents were applied by using following methods**

All three methods, namely seed soaking, pre-inoculation and post-inoculation, were adopted based on the procedure described by Kshirsagar and Deore (2020).

**2.1.1 Seed soaking in bioagents**

In this method, fifteen healthy papaya seeds per treatment were soaked in the biocontrol agent culture for 1 hour in a sterilized beaker. The treated seeds were then sown in plastic polythene bags containing a steam-sterilized mixture of soil, sand and compost in a 2:1:1 ratio to raise seedlings in an insect-proof screen house. Fifty days old seedlings, ten per treatment, were sap-inoculated with PRSV at the 6 to 8 leaf stage, and observations were recorded at 15 days intervals.

**2.1.2 Pre inoculation method**

In the pre-inoculation method, fifty days old seedlings, with ten seedlings per treatment at 6 to 8 leaf stage, were sprayed with the bioagent culture filtrate. 72 hours after spraying, the seedlings were sap-inoculated with PRSV extract, and observations were recorded at 15-day intervals.

**2.1.3 Post inoculation treatment**

In the post-inoculation method, fifty-day-old seedlings, with ten seedlings per treatment at 6 to 8 leaf stage, were sap-inoculated with PRSV extract. Seventy-two hours after inoculation, the inoculated seedlings were sprayed with the test biocontrol agent culture filtrate. Observations were recorded at 15-day intervals after inoculation.

**Observed parameters**

Observation on incubation period, disease incidence and reaction of PRSV and effect on plant height in papaya were recorded.

i) Incubation period i.e., number of days required to produce the symptoms after inoculation.

ii) Per cent disease incidence (PDI) @ 60 days after inoculation and calculated using the following formula as given by Chaing *et al*. (2017).

Number of infected plants

PDI = -------------------------------------------------- x 100

Total number of plants inoculated

iii) Symptom reactions of PRSV infected papaya plantssuch as vein clearing (Vc), chlorosis (C), mosaic (Mo), mild mosaic (MMo), blistering (Bl), leaf distortion (Ld), shoe stringing (Ss) and necrosis (N) were recorded at fifteen days intervals.

iv) Effect on plant height (cm) was measured at 60 DAI and the percentage increase or decrease in plant height was calculated based on bioagents treatments, relative to the inoculated untreated control, using the following formula.

T - C

Increase/ decrease in plant height (%) = --------------- x 100

T

Where, T = Plant height in treated plants

C = Plant height in inoculated untreated plants

**2.2 Statistical analysis**

The data obtained in all of the experiments (*in vitro* and *in vivo*) was subjected to the statistically analysed (Panse and Sukhatme, 1978). The per cent values was transformed intoarc sine values. The standard error (SE ±) and critical difference (C.D.) was computed at level P=0.01 and P=0.05, respectively for *in vitro* and *in vivo* experiments and interpreted results

**3. RESULTS AND DISCUSSION**

**3.1 Effect of bioagents on incubation period**

The results on the effect of bioagents on the incubation period were presented in Table 1 and Fig. 1. They revealed that the bioagents exhibited variations in their effects on the incubation period of PRSV, depending on the method and timing of application relative to inoculation. In the seed soaking method, the maximum incubation period was recorded with *Bacillus subtilis* (22 days), which was on par with *Pseudomonas fluorescens* (21 days), followed by *Streptomyces californicus* treatment (20 days). In the pre-inoculation method, the highest incubation period was recorded with *Bacillus subtilis* (24 days), which was on par with *Pseudomonas fluorescens* (23 days), followed by *Streptomyces californicus* treatment (22 days). In the post-inoculation method, the maximum incubation period was recorded with *Bacillus subtilis* (23 days), which was on par with *Pseudomonas fluorescens* (22 days), followed by *Streptomyces californicus* treatment (20 days). From these results, it was concluded that in all three methods *viz*., seed soaking, pre-inoculation and post-inoculation, all bioagent treatments effectively extended the incubation period of PRSV. However, among them, *Bacillus subtilis* and *Pseudomonas fluorescens* were found to be the most effective bioagents in extending the incubation period and delayed the symptom expression.

Several earlier studies reported similar findings. Raupach *et al.* (1996) affirmed that seed treatment with *Pseudomonas fluorescens* strain 89B-27 and *Serratia marcescens* strain 90-166 reduced the number of *Cucumber mosaic virus*-infected plants and delayed the development of symptoms in cucumber and tomato. Latake and Borkar (2017) studied the efficacy of actinomycetes as a seed treatment against CMV infection and found that isolate 21 had the longest incubation period of 22 days, followed by isolate 20, which had an incubation period of 20 days. In the spray treatment, the highest incubation period was observed in isolate 21 at 26 days, followed by isolate 13 at 15 days.

**3.2 Effect of bioagents on PRSV disease incidence**

The results on effect of bioagents on PRSV disease incidence were presented in Table 2 and Fig. 2 revealed that,result of seed soaking in bioagents revealed that there was minimum PRSV incidence was in *Pseudomonas fluorescens* (66.66%) compared to control followed by Biomix (70.00%), *Bacillus subtilis* with (73.33%), *Streptomyces californicus* (76.66%) and Pink pigmented facultative methylotrophs (83.33%). In pre inoculation method, the most effective treatments were *Pseudomonas fluorescens* (60.00%) followed by *Bacillus subtilis* (66.66%), *Streptomyces californicus* (70.00%), Biomix (73.33%) and Pink pigmented facultative methylotrophs (76.66%). Whereas, in post inoculation method, minimum per cent disease incidence was recorded in *Pseudomonas fluorescens* (63.33%) followed by *Bacillus subtilis* with (70.00%), *Streptomyces californicus* (73.33%) Biomix (76.66%) and Pink pigmented facultative methylotrophs (80.00%).

The results of the study reveal a notable variation in the per cent of disease incidence of PRSV based on the method and time of application. Among all the treatments evaluated, *Pseudomonas fluorescens*, *Bacillus subtilis* and *Streptomyces californicus* emerged as the most effective in reducing disease incidence, recording the lowest percentages. This indicates the potential efficacy of these biocontrol agents in mitigating the impact of PRSV, regardless of the method employed.

Several earlier studies have reported similar findings. Shoman *et al.* (2003) studied the effects of four rhizosphere microorganisms *Bacillus globisporus*, *Candida glabrata*, *Pseudomonas fluorescens* and *Streptomyces gibsonii* on inducing resistance to TNV through soil and foliar treatments. In Soil treatment results indicated that *Bacillus globisporus* was the most effective, achieving 59.20% inhibition, followed by *Candida glabrata* (49.30%) and *Pseudomonas fluorescens* (47.70%). In foliar treatments, *Streptomyces gibsonii* showed the highest inhibition at 97.20%, followed by *Pseudomonas fluorescens* (91.50%) and *Bacillus globisporus* (75.20%). Abd El-Shafi and Hussein (2012) evaluated the *in vivo* antiphytoviral activities of *Bacillus firmus*, *Bacillus subtilis* and their combination against Zucchini Yellow Mosaic Potyvirus (ZYMV) in squash plants. In *in vivo* conditions, 24 hours post inoculation, the mixture of both provided the highest inhibition (90.00%). While *Bacillus firmus* and *Bacillus subtilis* each achieved 80% inhibition. Barakat *et al. (*2012) investigated the efficiency of four bacterial strains (*Bacillus megatherium, Bacillus polymyxa, Zymomonas mobilis* ATCC 31823, and *Zymomonas mobilis* ATCC 10988) against *Watermelon mosaic virus-2* (WMV-2) infection to stimulate systemic acquired resistance (SAR) in watermelon plants and resulted that among these *Zymomonas mobilis* ATCC 31823 performed best (34.00% DS, 61.70% infection, 38.30% RI), followed by *Bacillus megatherium* (39.00% DS, 64.50% infection, 35.50% RI).

**3.3 Symptom** **reactions of PRSV to bioagents**

The results on symptom reactions of PRSV to bioagents were mentioned in Table 3 and revealed that, soaking seed in bioagents, extended incubation period and reported vein clearing chlorosis, mosaic and mild leaf distortions at 30 DAI in all treatments exhibited except control. at 45 DAI, the symptoms were progressed to chlorosisin *Pseudomonas fluorescens*, *Bacillus subtilis* and *Streptomyces californicus.* However, they further progressed to mosaic in *Trichoderma asperrellum,* Biomixand *Verticillum lecanii*. Rest all the treatments exhibited severe reactions like blistering, severe leaf distortion and shoe stringing. At 60DAI, *Pseudomonas fluorescens*, *Bacillus subtilis* and *Streptomyces californicus* were found to effective showed minimum symptoms as vein clearing, chlorosis, mosaic and mild leaf distortion followed by *Trichoderma asperrellum* and *T. harzianum* showed vein clearing, chlorosis, mosaic, mild leaf distortion and blistering on leaves.

In the pre-inoculation method, at 30 DAI, all treatments exhibited vein clearing symptoms except for *Pseudomonas fluorescens*, *Bacillus subtilis*, and *Streptomyces californicus*, which showed no symptoms. At 45 DAI, *Bacillus subtilis* was the most effective, showing minimal vein clearing, while in the other treatments, symptoms progressed to chlorosis in *Pseudomonas fluorescens*, *Streptomyces californicus*, Biomix, *Trichoderma asperellum* and *Verticillium lecanii*. The symptoms further developed into mild mosaic patterns in *Trichoderma harzianum*, PPMF, *Metarhizium anisopliae*, and *Beauveria bassiana*. In the control group, the symptoms advanced to blistering on leaves and shoestring deformities. By 60 DAI, *Bacillus subtilis* remained the most effective, showing the least severe symptoms, followed by *Pseudomonas fluorescens* and *Streptomyces californicus*, compared to the control. In the post inoculation method, at 30 DAI, all treatments exhibited vein clearing symptoms, except for the control, which showed vein clearing, chlorosis, mosaic patterns and mild leaf distortions. At 45 DAI, *Bacillus subtilis*, *Pseudomonas fluorescens* and *Streptomyces californicus* were found to be the most effective, showing only vein clearing and chlorosis. In the other treatments, symptoms progressed to mosaic patterns in Biomix, *Trichoderma asperellum*, PPMF, *Verticillium lecanii* and *Metarhizium anisopliae*. The symptoms further advanced to mild leaf distortion in *Trichoderma harzianum* and *Beauveria bassiana*, while in the control group, they developed into severe leaf distortion, blistering on leaves, and shoestring symptoms. By 60 DAI, *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Streptomyces californicus* remained effective, showing minimum symptoms compared to the control.

Similar findings were reported by Raupach *et al.* (1996), who affirmed that seed treatment with *Pseudomonas fluorescens* strain 89B-27 and *Serratia marcescens* strain 90-166 reduced the number of cucumber mosaic virus-infected plants and delayed the development of symptoms in cucumber and tomato.

**3.4 Effect of bioagents on papaya plant height**

The results on the effect of bioagents on papaya plant height were presented in Table 4 and Fig. 3. They revealed that, in the seed soaking in bioagents method at 60 DAI, the maximum increase in plant height was recorded with *Pseudomonas fluorescens* (5.67 cm), followed by *Bacillus subtilis* (4.73 cm), Biomix (4.60 cm), pink pigmented facultative methylotrophs (4.47 cm), *Streptomyces californicus* (4.40 cm), *Trichoderma asperellum* (4.16 cm), *Verticillium lecanii* (3.80 cm), *Metarhizium anisopliae* (3.63 cm) and *Trichoderma harzianum* (3.36 cm). However, the minimum increase in height was recorded in *Beauveria bassiana* (3.10 cm), followed by the untreated virus inoculated control (2.40 cm).

In the pre inoculation method at 60 DAI, the maximum increase in plant height was recorded with *Bacillus subtilis* (6.83 cm), followed by *Pseudomonas fluorescens* (5.76 cm), pink pigmented facultative methylotrophs (5.47 cm), *Streptomyces californicus* (5.14 cm), Biomix (5.06 cm), *Trichoderma harzianum* (4.70 cm), *Trichoderma asperellum* (4.00 cm), *Verticillium lecanii* (3.96 cm), and *Metarhizium anisopliae* (3.17 cm). However, the minimum increase in height was recorded in *Beauveria bassiana* (3.14 cm), followed by the untreated virus inoculated control (2.57 cm).

In the post inoculation method at 60 DAI, the maximum increase in plant height was recorded with *Bacillus subtilis* (6.57 cm), followed by *Pseudomonas fluorescens* (5.74 cm), *Streptomyces californicus* (5.36 cm), Biomix (4.84 cm), pink pigmented facultative methylotrophs (4.77 cm), *Trichoderma asperellum* (4.50 cm), *Trichoderma harzianum* (4.10 cm), *Metarhizium anisopliae* (3.46 cm), and *Verticillium lecanii* (3.30 cm). However, the minimum increase in height was recorded in *Beauveria bassiana* (3.13 cm), followed by the untreated virus-inoculated control (2.13 cm).

Several earlier studies reported similar findings. Maha et al. (2008) investigated the effects of *Streptomyces chibaensis* on inducing resistance in banana plants against Banana bunchy top virus (BBTV) using either a cell-free filtrate or a spore suspension, applied both before and after virus inoculation. The highest plant growth parameters, such as pseudostem height, number of leaves, and leaf area, were observed in the Fpre treatment and its control (FHC), which showed no incidence of the virus. Similarly, Li et al. (2019) studied the effects of *Streptomyces pactum* Act12 on tomato plant biomass and found that plant height in the Act12 treatment was 16.1% higher than in the control.

**4 CONCLUSIONS**

From the present study, it can be concluded that, none of the bioagent was effective in inducing complete resistance in papaya against papaya ring spot virus (PRSV), but among all the methods such as seed soaking in bioagents, pre and post inoculation of bioagents, the *Bacillus subtilis* and *Pseudomonas fluorescens* was found to be most effective in inducing disease resistance by extending incubation period, recorded less disease incidence and extend symptom expression and expressing mild reactions to PRSV virus. Though, all the bioagents enhanced plant height of papaya but the bioagents like *Bacillus subtilis, Pseudomonas fluorescens* and *Streptomyces californicus* were found to be effective and the mode of actions of bioagents in inducing resistance against PRSV needs to be investigated further as several morphological and biochemical changes within the host plants were probably the reason for such defense responses.

**REFERENCES**

Abdel-Shafi, S. E. H. A. M., & Hussein, Y. A. S. S. E. R. (2012). Biological control of Zucchini Yellow Mosaic Potyvirus (ZYMV) by *Bacillus firmus* and *Bacillus subtilis* in squash plants. *The Egyptian Journal of Botany.* 52(1), 99–115.

Anonymous. (2024). *Indiastat*. <https://www.indiastat.com/table/agriculture/area-production-productivity-papaya-india-1950-195/14865>.

Barakat, O. S., Goda, H. A., Mahmoud, S. M., & Emara, K. S. (2012). Induction of systemic acquired resistance in watermelon against watermelon mosaic virus-2. *Arab Journal of Biotechnology.* 15(2), 23–44.

Chiang, K., Liu, H., & Bock, C. H. (2017). A discussion on disease severity index values: Warning on inherent errors and suggestions to maximize accuracy. *Annals of Applied Biology.* 171, 139–154.

Kessmann, H., Staub, T., Ligon, J., Oostendorp, M., & Ryals, J. (1994). Activation of systemic acquired disease resistance in plants. *European Journal of Plant Pathology.* 100, 359–369.

Kshirsagar, H., & Deore, P. (2020). Studies on induced resistance by chemicals against papaya ring spot virus (PRSV) in papaya. *International Journal of Current Microbiology & Applied Sciences.* 9(11), 2074–2083.

Latake, S. B., & Borkar, S. G. (2017). Characterization of marine actinomycete with antiviral activity against cucumber mosaic virus. *Current Science.* 113(7).

Li, Y., Guo, Q., Li, Y., Sun, Y., Xue, Q., & Lai, H. (2019). *Streptomyces pactum* Act12 controls tomato yellow leaf curl virus disease and alters rhizosphere microbial communities. *Biology and Fertility of Soils*. <https://doi.org/10.1007/s00374-019-01339-w>.

Maha, A. H., Husseiny, S. M., Salama, M. I., Hanan, A. N. E., & Shimaa, M. A. (2008). Reduction in banana bunchy top virus infection rate by using *Streptomyces chibaensis* filtrate. *Pakistan Journal of Biotechnology.* 5(1/2), 51–61.

Mishra, M., Rames, C., & Sangeeta, S. (2007). Genome mapping and molecular breeding in plants. In *Fruits and Nuts* (pp. 343–351). Heidelberg: Springer-Verlag.

Raupach, G. S., Liu, L. I., Murphy, F., Tuzun, S., & Kloepper, J. W. (1996). Induced systemic resistance in cucumber and tomato against cucumber mosaic cucumovirus using plant growth-promoting rhizobacteria (PGPR). *Plant Disease.* 80(8), 891–894.

Sharma, S. K., & Tripathi, S. (2014). Papaya ringspot virus-P: Overcoming limitations of resistance breeding in *Carica papaya* L. In *Plant Virus Host Interaction* (pp. 177–194). Academic Press.

Shoman, S. A., Abd-Allah, N. A., & El-Baz, A. F. (2003). Induction of resistance to tobacco necrosis virus in bean plants by certain microbial isolates. Egyptian Journal of Biology. 5, 10-18.

**Table 1.** **Effect of bioagents on incubation period of PRSV**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Tr. no.** | **Treatments** | **Incubation period (days) \*** | | |
| **Seed soaking in chemicals** | **Pre inoculation of chemicals** | **Post inoculation of chemicals** |
| **T1** | *Trichoderma asperellum* | 19 | 20 | 20 |
| **T2** | *Trichoderma harzianum* | 19 | 20 | 19 |
| **T3** | *Verticellium lecanii* | 18 | 19 | 18 |
| **T4** | *Bacillus subtilis* | 22 | 24 | 23 |
| **T5** | *Pseudomonas fluorescens* | 21 | 23 | 22 |
| **T6** | Pink pigmented facultative methylotrophs | 18 | 21 | 20 |
| **T7** | *Streptomyces californicus* | 20 | 22 | 20 |
| **T8** | *Metarhizium anisopliae* | 17 | 19 | 18 |
| **T9** | *Beauveria bassiana* | 17 | 18 | 18 |
| **T10** | Biomix | 19 | 21 | 20 |
| **T11** | Untreated virus inoculated control | 15 | 15 | 15 |
| **S. E. ±** | | **0.59** | **0.52** | **0.57** |
| **C. D. (P=0.01)** | | **1.74** | **1.55** | **1.68** |

\*: Mean of three replications

**Table 2. Effect of bioagents on PRSV disease incidence at 60 days after inoculation**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Tr. no.** | **Treatments** | **PRSV incidence\***  **(%)** | | |
| **Seed soaking in chemicals** | **Pre inoculation of chemicals** | **Post inoculation of chemicals** |
| **T1** | *Trichoderma asperellum* | 86.66  **(68.57)** | 80.00  **(63.43)** | 90.00  **(71.56)** |
| **T2** | *Trichoderma harzianum* | 90.00  **(71.56)** | 83.33  **(65.90)** | 86.66  **(68.57)** |
| **T3** | *Verticellium lecanii* | 93.33  **(75.03)** | 86.66  **(68.57)** | 90.00  **(71.56)** |
| **T4** | *Bacillus subtilis* | 73.33  **(58.90)** | 66.66  **(54.73)** | 70.00  **(56.78)** |
| **T5** | *Pseudomonas fluorescens* | 66.66  **(54.73)** | 60.00  **(50.76)** | 63.33  **(52.73)** |
| **T6** | Pink pigmented facultative methylotrophs | 83.33  **(65.90)** | 76.66  **(61.11)** | 80.00  **(63.43)** |
| **T7** | *Streptomyces californicus* | 76.66  **(61.11)** | 70.00  **(56.78)** | 73.33  **(58.90)** |
| **T8** | *Metarhizium anisopliae* | 93.33  **(75.03)** | 90.00  **(71.56)** | 96.66  **(79.46)** |
| **T9** | *Beauveria bassiana* | 96.66  **(79.46)** | 93.33  **(75.03)** | 96.66  **(79.46)** |
| **T10** | Biomix | 70.00  **(56.78)** | 73.33  **(58.90)** | 76.66  **(61.11)** |
| **T11** | Untreated virus inoculated control | 100.00  **(90.00)** | 100.00  **(90.00)** | 100.00  **(90.00)** |
| **S. E. ±** | | **0.58** | **0.55** | **0.54** |
| **C. D. (P=0.01)** | | **1.72** | **1.64** | **1.62** |

\*Mean of three replications, figures in parentheses arc sine values, PDI: Per cent disease incidence

**Table 3. Reactions of PRSV to bioagents in papaya Cv. Red Lady at different intervals**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Tr. no.** | **Treatments** | **Seed soaking method** | | | | **Pre inoculation method** | | | | **Post inoculation method** | | | | |
| **15 DAI** | **30 DAI** | **45 DAI** | **60 DAI** | **15 DAI** | **30 DAI** | **45 DAI** | **60 DAI** | **15 DAI** | **30 DAI** | **45 DAI** | **60 DAI** |
| **T1** | *Trichoderma asperellum* | - | Vc | Vc, C, M | Vc, C, M, Mld, B | - | Vc | Vc, C | Vc, C, Mm, Ld, B | - | Vc | Vc, C, M | Vc, C, M, Ld, B  , |
| **T2** | *Trichoderma harzianum* | - | Vc | Vc, C, M, Mld | Vc, C, M, Mld, B | - | Vc | Vc, C,  Mm | Vc, C, Mm, Ld, B | - | Vc | Vc, C, M, Mld | Vc, C, M, Sld, B |
| **T3** | *Verticellium lecanii* | - | Vc | Vc, C, M | Vc, C, M, Sld, B | - | Vc | Vc, C | Vc, C, Mm, Mld, B | - | Vc | Vc, C, M | Vc, C, M, Ld, B |
| **T4** | *Bacillus subtilis* | - | Vc | Vc, C | Vc,C, M, Mld | - | - | Vc, | Vc,C, Mm | - | Vc | Vc, C | Vc,C, M, Mld |
| **T5** | *Pseudomonas fluorescens* | - | Vc | Vc, C | Vc,C, M, Mld | - | - | Vc, C | Vc,C, Mm, Mld | - | Vc | Vc,C | Vc,C, M, Mld |
| **T6** | Pink pigmented facultative methylotrophs | - | Vc | Vc, C, M,  B | Vc, C, M, Sld, B | - | Vc | Vc, C,  Mm | Vc, C, Mm, Mld, B | - | Vc | Vc, C, M | Vc, C, M, Sld, B |
| **T7** | *Streptomyces californicus* | - | Vc | Vc, C | Vc,C, M, Mld | - | - | Vc, C | Vc,C, Mm, Mld | - | Vc | Vc,C | Vc,C, M, Mld |
| **T8** | *Metarhizium anisopliae* | - | Vc | Vc, C, M,  B | Vc, C, M, Sld, B | - | Vc | Vc, C,  Mm | Vc, C, Mm, Mld, B | - | Vc | Vc, C, M | Vc, C, M, Ld, B |
| **T9** | *Beauveria bassiana* | - | Vc | Vc, C, M, Mld | Vc, C, M, Sld, B | - | Vc | Vc, C,  Mm | Vc, C, Mm, Mld, B | - | Vc | Vc, C, M, Mld | Vc, C, M, Sld, B |
| **T10** | Biomix | - | Vc | Vc, C, M | Vc, C, M, Sld, B | - | Vc | Vc, C | Vc, C, Mm, Mld, B | - | Vc | Vc, C, M | Vc, C, M, Sld, B |
| **T11** | Untreated virus inoculated control | - | Vc, C, M, MLd | Vc,,C, M, Sld,B | Vc,, C, M,Sld, B,Ss | - | Vc, C, M, MLd | Vc,,C, M, Sld,B | Vc,, C, M,Sld, B,Ss | - | Vc, C, M, MLd | Vc,,C, M, Sld, B | Vc,, C, M, Sld, B,Ss |

\*DAI : Days After Inoculation, - : No any viral symptoms, Vc: Vein clearing, C: Chlorosis, M: Mosaic, Mm: Mild mosaic, B: Blistering, Mld: Mild leaf distortion, Sld: Severe leaf distortion, Ss: Shoe string

**Table 4. Effect of bioagents treatments and PRSV inoculation on plant height in papaya cv. Red Lady**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Tr. no.** | **Treatments** | **Papaya plant height at 60 DAI** | | | | | |
| **Seed soaking in bioagents** | | **Pre inoculation of bioagents** | | **Post inoculation of bioagents** | |
| **Increase in Plant height** | **PIH**  **(%)** | **Increase in Plant height** | **PIH**  **(%)** | **Increase in Plant height** | **PIH**  **(%)** |
| **T1** | *Trichoderma asperellum* | 4.16 | 42.30 | 4.00 | 35.75 | 4.50 | 52.66 |
| **T2** | *Trichoderma harzianum* | 3.36 | 28.57 | 4.70 | 45.31 | 4.10 | 48.04 |
| **T3** | *Verticellium lecanii* | 3.8 | 36.84 | 3.96 | 35.10 | 3.30 | 35.45 |
| **T4** | *Bacillus subtilis* | 4.73 | 49.26 | 6.83 | 25.91 | 6.57 | 67.57 |
| **T5** | *Pseudomonas fluorescens* | 5.67 | 57.67 | 5.76 | 55.38 | 5.74 | 62.89 |
| **T6** | Pink pigmented facultative methylotrophs | 4.47 | 46.30 | 5.47 | 53.01 | 4.77 | 55.34 |
| **T7** | *Streptomyces californicus* | 4.4 | 45.45 | 5.14 | 50.00 | 5.36 | 60.26 |
| **T8** | *Metarhizium anisopliae* | 3.63 | 33.88 | 3.17 | 18.92 | 3.46 | 38.43 |
| **T9** | *Beauveria bassiana* | 3.1 | 22.58 | 3.14 | 18.15 | 3.13 | 31.94 |
| **T10** | Biomix | 4.6 | 47.82 | 5.06 | 49.20 | 4.84 | 55.99 |
| **T11** | Untreated virus inoculated control | 2.4 | - | 2.57 | - | 2.13 | - |
|  | **S. E. ±** | **0.48** | **-** | **0.52** | **-** | **0.55** | **-** |
| **C. D. (P=0.01)** | **1.44** | **-** | **1.56** | **-** | **1.64** | **-** |

DAI : Days after inoculation; PIH : Per cent increase in plant height over control

**Fig. 1 Effect of bioagents on incubation period of PRSV**

**Fig. 2 Effect of bioagents on incidence of PRSV at 60 Days after inoculation**

**Fig. 3 Effect of bioagents on increase in papaya plant height at 60 DAI**