Generation of a Bio-intensive Strategy using Chitosan Formulations and *Ampelomyces quisqualis* for the Management of Powdery Mildew of Grapes

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Abstract

Powdery mildew caused by *Erysiphe necator* is one of the most important diseases of grapevine in India. It affects the grape production by causing significant reduction in yield and quality of grapes. Chitosan is a deactivated derivative of chitin and the efficacy of three chitosan formulations, Chitosan fulvate @ 2ml/L, Chitosan oligosaccharides @ 2ml/L, Chitosan @ 4ml/L were evaluated against powdery mildew. The physiological loss in weight of berries was also assessed. During the fruiting seasons of 2018-2019 and 2019-2020, chitosan formulations were evaluated both as solo treatments and in alternation with *Ampelomyces quisqualis* @ 5ml/L. All Chitosan formulations effectively inhibited powdery mildew on leaves and berries when compared to untreated control. Among solo treatments, chitosan exhibited lower PDI (17.76 and 5.29) on leaves than untreated control (24.28 and 13.12) during 2018-2019 and 2019-2020 respectively. In case of bunches, similar trend was observed with PDI 23.39 and 9.90 against untreated control 30.31 and 17.18 in both seasons. However, the formulations were found more effective when used in alternation with *Ampelomyces quisqualis*. During 2018-2019 and 2019-2020, Chitosan / *Ampelomyces quisqualis* treatment recorded significantly lower PDI of powdery mildew on leaves i.e.12.55 and 4.77 than the untreated control with PDI 24.28 and 13.22 respectively. In case of bunches, treatment Chitosan / *Ampelomyces quisqualis* showed the lowest disease severity with PDI 19.28 and 8.98 against untreated control with PDI 31.60 and 17.18 in both the seasons respectively. The applications of formulation containing chitosan alternated with sprays of *Ampelomyces quisqualis* (5ml/L) showed least disease as well physiological loss in weight of grapes. All chitosan formulations were effective in minimizing physiological loss in weight of grapes as compared to control. Among all treatments, Chitosan / *Ampelomyces quisqualis* treatment recorded the lowest PLW i.e.13.94 and 13.90, whereas untreated control showed the highest PLW i.e. 15.85 and 15.87 during both seasons respectively. Results showed that chitosan can be effectively used in powdery mildew management, either alone or in alternation with the bio-formulation of *Ampelomyces quisqualis*.

Keywords

Chitosan, Powdery mildew, Grapes, *Erysiphe necator, Ampelomyces quisqualis*

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Introduction

Grape is an important commercial fruit crop and is most widely cultivated in temperate, sub-tropical and tropical regions of the world. This crop occupies fifth position amongst fruit crops in India with a production of 1.21 million tonnes. The area under grape cultivation in India is 137 thousand hectares with production of 2951 thousand MT (Anonymous, 2019). The commercial grape varieties are susceptible to several diseases. Among these, the powdery mildew caused due to *Erysiphe necator* Schwein. (syn. *Uncinula necator* Schwein.), is one of the most severe diseases in all grape growing areas (Iriti et al., 2011, Sharma et al., 2017).

The malady is characterized by white powder like growth on the leaves, immature shoots and young berries and is responsible for affecting the quality and yield of grapes worldwide (Gadoury et al., 2012). Fungicides are the major tools in the control of the disease [23] but non-rational use of fungicides leads to health risk, both to the environment and human being. The congruent effect of the rising environmental risk due to xenobiotics along with the selection of pathogen strains resistant to chemicals and the cost of traditional pesticide treatments have prompted the studies on alternative strategies in crop protection. Moreover, in the recent times, a lot of emphasis is given on minimum pesticide residues in grapes, especially in export grapes to the European Union and the search for alternatives became imperative. Alternative or low profile compounds like ozonated water (Fujiwara and Fujii, 2002) chlorine dioxide (Sharma et al., 2017) etc. are used in the post veraison stage to control powdery mildew disease of grapes.

Chitosan (β-1, 4-D-glucosamine polymer) is a natural, safe, and cheap biopolymer produced from chitin, the major constituent of arthropods exoskeleton and fungal cell walls and the second renewable carbon source after lignocellulosic biomass (Barber et al 1989). Chitosan oligosaccharide is a mixture of oligomers of D-glucosamine. The plants’ response to chitin, chitosan, and derived oligosaccharides depends on the acetylation degree of these compounds which indicates possible biocontrol regulation of plant immune system (Akiyama et al., 1995, Cord-Landwehr et al., 2016, Li et al., 2016) and the degree of polymerization (Walker-Simmons and Ryan 1984, Li et al., 2016) of these compounds. Chitosan and its oligomers have been used for a plethora of reasons in crop husbandry viz. as biopesticides, biofertilisers, elicitor for induction of natural resistance of plant, seed coating formulations and agricultural film (Ha and Huang 2007, El Hadrami et al., 2010; Trouvelot et al., 2014) due to its unique attributes such as biodegradability, nontoxicity, biocompatibility and fungicidal effect (Rinaudo, 2006, Kumar, 2000). Chitosan treatment increases plants tolerance to the attack of wide range of soil and foliar pathogens and induces root nodulation as well (Hamel et al., 2010).

 Keeping this at the backdrop, the efficacy of chitosan and oligo-chitosan formulations were assessed against grapevine powdery mildew disease.

Materials and Methods

The efficacy of three chitosan formulations provided by the AuraphyllInnoventures India Pvt. Limited viz. Chitosan fulvate (Chit’o’sun) @ 2mL/L, Chitosan oligosaccharides (Chitang) @ 2mL/L and Chitosan (Vilasae) @ 4mL/L were evaluated against powdery mildew infection on grape leaves and berries of cv. Manik Chaman. The trial was conducted at an experimental vineyard of ICAR-National Research Centre
for Grapes, Pune (latitude 18.31N, longitude 73.55 E) during fruiting season 2018-2019 and 2019-2020.

The trial was set up as a Randomised block design with four replications, with 10 vines (an experimental plot) per treatment in each block. Chitosan formulations were evaluated both as solo treatments and in alternation with Ampelomyces quisqualis @ 5ml/L. A total of 8 treatments were taken into account. Applications were given at 7 days interval from the beginning of grape susceptibility to powdery mildew until the completion of veraison. Treatment with sprays of Ampelomyces quisqualis @ 5ml/L and untreated control were used as check.

| Sr. No. | Treatments                              | Dose       | Total no. of applications |
|---------|-----------------------------------------|------------|---------------------------|
| 1       | Chitosan fulvate                        | 2 ml       | 5                         |
| 2       | Chitosan oligosaccharides               | 2 ml       | 5                         |
| 3       | Chitosan                                | 4 ml       | 5                         |
| 4       | Ampelomyces quisqualis                  | 5 ml       | 5                         |
| 5       | Chitosan fulvate / Ampelomyces quisqualis | 2 ml / 5 ml | 3/2                       |
| 6       | Chitosan oligosaccharides /Ampelomyces quisqualis | 2 ml / 5 ml | 3/2                       |
| 7       | Chitosan /Ampelomyces quisqualis        | 4 ml / 5 ml | 3/2                       |
| 8       | Untreated Control                       | --         | --                        |

To avoid spray drift to neighbouring plots, sprays were carried out with knapsack sprayers. Symptoms were assessed weekly on leaves and bunches, by visual inspections and disease severity were evaluated on a scale of 0–5, where 0 = no symptoms; 1 = 1–10%; 2 = 11–25%; 3 = 26–50%; 4 = 51–75%; and 5 = 76–100% of infected leaf areas or infected berries per bunch, respectively (Horsfall and Heuberger, 1942). Data regarding disease severity were processed according to McKinney, 1923, in order to calculate the percentage of disease severity (PDI):

\[ PDI = \frac{\text{sum of numerical ratings}}{\text{No. of leaves/bunches observed}} \times \frac{100}{\text{Maximum rating}} \]

The physiological loss in weight of berries was also assessed. Weight of bunches were recorded for all treatments at 24 hour intervals for first 5-7 days at room temperature. The percent loss of weight over initial weight was calculated mathematically for grapes in each replicate.

**Statistical analysis**

The data were analyzed in RBD design with analysis of variance (ANOVA) using SAS (ver. 9.3; SAS Institute Inc., Cary, North Carolina, USA). The percentage data was arcsine-transformed before analysis.

**Results and Discussion**

**Bio-efficacy of chitosan formulations on grapes leaves**

In 2018-2019 and 2019-2020, first disease observations on leaves in experimental plot were recorded on 15\textsuperscript{th} January 2019 and 02\textsuperscript{nd} January 2020 respectively (Table 1). The disease severity increased slowly from first to last observation. The PDI of powdery mildew on the leaves were gradually increased from 22.34 to 24.28 and 11.29 to 13.12 in the unsprayed control respectively. Chitosan formulations effectively inhibited powdery mildew on leaves when compared to untreated control. However, the formulations
were found more effective when used in alternation with *Ampelomyces quisqualis* (Table 1). In the last observation recorded on 12.2.19, Chitosan / *Ampelomyces quisqualis* treatment recorded significantly lower PDI of powdery mildew on leaves *i.e.* 12.55 than the untreated control (24.28). The alternation of chitosan fulvate, chitosan oligosaccharides and Chitosan with *Ampelomyces quisqualis* were statistically at par with each other with PDI of 14.22, 13.82 and 12.55 respectively. In case of solo treatment, chitosan exhibited lower PDI (17.76) than other treatments but all the solo treatments were statistically on par with each other.

In 2019-2020, similar trend was observed as previous trial. All treatments performed significantly superior over untreated control. Chitosan / *Ampelomyces quisqualis* showed lower PDI (4.77) than other treatments during last observation. All the treatments except untreated control were statistically at par with each other. As the disease incidence was negligible on leaves during 2019-20, less variation in the result of different treatments were observed. The percent disease intensity on leaves in untreated control plot was 13.12.

In case of bunches, similar trend was observed (Table 2). The last observation recorded on 12.2.2019 showed that treatment Chitosan / *Ampelomyces quisqualis* recorded a significantly lower PDI of powdery mildew on bunches *i.e.* 19.28 than the untreated control (31.60). All the treatments were significantly superior over untreated control. Treatments Chitosan / *Ampelomyces quisqualis* and Chitosan oligosaccharides / *Ampelomyces quisqualis* were at par with each other and significantly superior over other treatments. Treatment *Ampelomyces quisqualis* and Chitosan fulvate / *Ampelomyces quisqualis* were at par with each other with a PDI values of 20.99 and 21.39 respectively.

In 2019-2020, the last observation recorded on 28.01.2020 showed that treatment Chitosan / *Ampelomyces quisqualis* recorded a significantly lower PDI of powdery mildew on bunches *i.e.* 8.98 than the untreated control (17.18). All the treatments were significantly superior over untreated control. Chitosan / *Ampelomyces quisqualis*, Chitosan oligosaccharides / *Ampelomyces quisqualis*, Chitosan fulvate / *Ampelomyces quisqualis* and Chitosan treatments were at par with each other. Treatment *Ampelomyces quisqualis* and Chitosan oligosaccharides were at par with each other.

**Physiological loss in weight (PLW)**

Results (Table 3) showed the significant differences among the treatments during the storage at ambient conditions. Control recorded higher PLW than all the treatments. By increasing the storage duration, the PLW also increased.

In 2018-2019, on third day of storage, physiological loss in weight (PLW) in control reached up to 13.73, whereas PLW in Chitosan / *Ampelomyces quisqualis* was significantly lesser (11.77) than untreated control. Chitosan treatment and Chitosan oligosaccharides / *Ampelomyces quisqualis* treatment registered with values of less than 5 per cent. On 4th day, PLW values were more than 5% in all treatments, but the untreated control had the highest value of 15.87. PLW value in Chitosan / *Ampelomyces quisqualis* treatment was only 13.94. Similar observations were also recorded in season 2019-2020. On third day of storage, physiological loss in weight (PLW) in control reached up to 14.04, whereas PLW in Chitosan / *Ampelomyces quisqualis* recorded significantly lesser (11.73) than untreated control. Remaining treatments also registered with values of less than 5 per cent.
Table 1: Effect of different chitosan formulations on powdery mildew severity in grapes leaves during 2018-2019 and 2019-2020

| Tr. No. | Tr. Name | PDI (2018-2019) | PDI (2019-2020) |
|---------|----------|-----------------|-----------------|
|         |          | 15/01/19 | 22/01/19 | 29/01/19 | 05/02/19 | 12/02/19 | 02/01/20 | 09/01/20 | 16/01/20 | 23/01/20 | 30/01/20 |
| 1       | Chitosan fulvate | 0.00 | (0.00) a | 10.10 (18.39) | d | 11.20 | (19.53)b | 11.30 | (19.62)b | 11.40 | (19.71) b | 0.00 | (0.00) a | 0.35 | (3.38) a | 0.4 | (3.55) a | 0.65 | (4.59) a | 0.95 | (5.58) a |
| 2       | Chitosan oligosaccharides | 0.00 | (0.00) a | 8.70 (17.15) | cd | 10.35 | (18.61)b | 10.50 | (18.76)b | 10.60 | (18.86) b | 0.00 | (0.00) a | 0.35 | (3.38) a | 0.5 | (4.04) a | 0.75 | (4.95) a | 0.95 | (5.58) a |
| 3       | Chitosan | 0.00 | (0.00) a | 6.70 (14.94) | bc | 9.00 (17.46) | b | 9.10 | (17.56)b | b | 9.30 (17.76) | 0.00 | (0.00) a | 0.20 | (1.81) a | 0.55 | (4.23) a | 0.55 | (4.20) a | 0.85 | (5.29) a |
| 4       | Ampelomyces quisqualis | 0.00 | (0.00) a | 5.45 (13.40) | ab | 5.80 | (13.86)a | 5.90 | (14.00)a | 6.15 (14.32) | a | 0.00 | (0.00) a | 0.25 | (2.01) a | 0.65 | (4.61) a | 0.70 | (4.22) a | 0.85 | (5.29) a |
| 5       | Chitosan fulvate / Ampelomyces quisqualis | 0.00 | (0.00) a | 5.30 (13.28) | ab | 5.75 | (13.85)a | 5.85 | (13.97)a | 6.05 (14.22) | a | 0.00 | (0.00) a | 0.30 | (2.72) a | 0.50 | (4.04) a | 0.70 | (4.22) a | 0.9 | (5.44) a |
| 6       | Chitosan oligosaccharides / Ampelomyces quisqualis | 0.00 | (0.00) a | 4.75 (13.55) | ab | 5.40 | (13.37)a | 5.55 | (13.56)a | 5.75 (13.82) | a | 0.00 | (0.00) a | 0.15 | (1.55) a | 0.40 | (3.55) a | 0.45 | (3.84) a | 0.75 | (4.96) a |
| 7       | Chitosan /Ampelomyces quisqualis | 0.00 | (0.00) a | 3.90 (11.90) | a | 4.35 | (11.93)a | 4.60 | (12.28)a | 4.80 (12.55) | a | 0.00 | (0.00) a | 0.35 | (3.36) a | 0.55 | (4.23) a | 0.65 | (4.59) a | 0.70 | (4.77) a |
| 8       | Untreated Control | 10.60 | (18.88) b | 14.60 | (22.34)e | 16.70 | (23.90)c | 16.90 | (24.08)c | 17.15 | (24.28)c | 17.15 | (24.28)c | 16.90 | (24.08)c | 17.15 | (24.28)c | 17.15 | (24.28)c | 16.90 | (24.08)c | 17.15 | (24.28)c |
|         | LSD (p=0.05) | 1.38 | 2.62 | 3.24 | 3.05 | 2.96 | 0.71 | 2.19 | 1.10 | 1.68 | 0.87 |

Means with the same letter are not significantly different. Fig. in the parenthesis show angular transform values.
Table.2 Effect of different chitosan formulations on powdery mildew severity on grapes bunches

| Tr. No. | Tr. Name                              | PDI (2018-2019) | PDI (2019-2020) |
|---------|---------------------------------------|-----------------|-----------------|
|         |                                       | 15/01/19        | 22/01/19        | 29/01/19 | 05/02/19 | 12/02/19 | 02/01/20 | 08/01/20 | 15/01/20 | 22/01/20 | 28/01/20 |
| 1       | Chitosan fulvate                       | 0.00            | 10.50           | 16.00   | 17.50   | 19.50   | 0.00     | 2.50     | 3.00     | 4.00     | 5.50     |
|         |                                       | (0.00) a        | (18.82) a       | (23.48) b| (24.62) c| (26.12) c| (0.00) a | (9.06) de| (3.54) a | (11.42) b| (13.51) b|
| 2       | Chitosan oligosaccharides              | 0.00            | 12.50           | 15.50   | 16.50   | 18.50   | 0.00     | 2.00     | 2.25     | 2.75     | 4.25     |
|         |                                       | (0.00) a        | (20.52) ab      | (23.10) b| (23.83) bc| (25.36) bc| (0.00) a | (8.13) dc| (4.02) a | (9.44) ab| (11.83) ab|
| 3       | Chitosan                              | 0.00            | 15.50           | 13.50   | 14.00   | 16.00   | 0.00     | 1.50     | 1.75     | 2.75     | 3.00     |
|         |                                       | (0.00) a        | (22.99) b       | (21.44) ab| (21.74) abc| (23.39) abc| (0.00) a | (6.93) bc| (4.23) a | (9.44) ab| (9.90) a  |
| 4       | Ampelomyces quisqualis                | 0.00            | 8.50            | 10.00   | 11.00   | 13.00   | 0.00     | 2.25     | 2.50     | 2.50     | 3.50     |
|         |                                       | (0.00) a        | (16.93) a       | (18.39) a| (19.17) ab| (20.99) ab| (0.00) a | (8.62) edc| (4.61) a | (9.05) ab| (10.64) ab|
| 5       | Chitosan fulvate / A. quisqualis       | 0.00            | 10.00           | 12.00   | 12.50   | 13.50   | 0.00     | 1.50     | 1.50     | 2.25     | 3.25     |
|         |                                       | (0.00) a        | (18.21) a       | (20.10) ab| (20.56) abc| (21.39) ab| (0.00) a | (6.97) bc| (4.04) a | (8.43) ab| (9.65) a  |
| 6       | Chitosan oligosaccharides / A. quisqualis | 0.00        | 9.00            | 10.00   | 10.50   | 12.00   | 0.00     | 1.25     | 1.75     | 2.00     | 2.75     |
|         |                                       | (0.00) a        | (17.43) a       | (18.05) a| (18.71) a| (20.14) a| (0.00) a | (6.21) b | (3.54) a | (7.79) a | (9.07) a  |
| 7       | Chitosan / A. quisqualis              | 0.00            | 8.50            | 9.50    | 10.00   | 11.00   | 0.00     | 0.40     | 1.25     | 1.75     | 2.50     |
|         |                                       | (0.00) a        | (16.67) a       | (17.89) a| (18.35) a| (19.28) a| (0.00) a | (3.61) a | (4.23) a | (7.29) a | (8.98) a  |
| 8       | Untreated Control                     | 12.50           | 24.50           | 25.00   | 25.50   | 27.50   | 3.00     | 3.25     | 5.25     | 7.5      | 8.75     |
|         |                                       | (20.60) b       | (29.66) c       | (29.96) c| (30.31) d| (31.60) d| (9.79) b | (10.29) e| (11.76) b| (15.67) c| (17.18) c|
|         | LSD (p=0.05)                          | **1.36**        | **4.16**        | **4.29**| **4.66**| **4.49**| **1.21** | **1.83** | **1.10** | **3.13** | **3.08** |

Means with the same letter are not significantly different. Fig. in the parenthesis show angular transform values.
Table 3: Effect of application of different chitosan formulations on physiological loss in weight of grapes

| Tr. No. | Tr. Name                  | 2018-2019 |       |       |       | 2019-2020 |       |       |       |
|---------|---------------------------|-----------|-------|-------|-------|-----------|-------|-------|-------|
|         |                           | 1st day   | 2nd day | 3rd day | 4th day | 1st day   | 2nd day | 3rd day | 4th day |
| 1       | Chitosan fulvate          | 1.50      | 3.59   | 5.07   | 7.14   | 2.26      | 3.62   | 4.36   | 6.22   |
|         |                           | (7.00) ab | (10.90) cd | (13.01) bcd | (15.49) cde | (8.63) a | (10.97) ab | (11.75) a | (14.42) ab |
| 2       | Chitosan oligosaccharides | 1.73      | 3.21   | 5.28   | 6.59   | 2.32      | 3.52   | 4.46   | 5.93   |
|         |                           | (7.56) cd | (10.32) bc | (13.27) cd | (14.86) abcd | (8.74) ab | (10.81) ab | (12.16) a | (13.99) a |
| 3       | Chitosan                  | 1.33      | 2.98   | 4.72   | 6.37   | 2.26      | 3.47   | 4.27   | 5.92   |
|         |                           | (6.61) a | (9.93) b | (12.54) abc | (14.61) ab | (8.63) a | (10.72) ab | (11.91) a | (14.08) a |
| 4       | Ampelomyces quisqualis    | 1.76      | 3.63   | 5.64   | 7.34   | 2.48      | 3.72   | 4.78   | 6.24   |
|         |                           | (7.61) d | (10.98) cd | (13.72) de | (15.79) de | (9.00) ab | (11.08) ab | (12.62) ab | (14.45) ab |
| 5       | Chitosan fulvate / Ampelomyces quisqualis | 1.74 | 3.46 | 5.19 | 6.88 | 2.41 | 3.59 | 4.40 | 6.11 |
|         |                           | (7.58) cd | (10.69) cd | (13.15) bcd | (15.19) bcde | (8.93) ab | (10.49) a | (12.11) a | (14.30) a |
| 6       | Chitosan oligosaccharides /Ampelomyces quisqualis | 1.32 | 2.89 | 4.63 | 6.32 | 2.29 | 3.31 | 4.24 | 5.85 |
|         |                           | (6.56) a | (9.76) b | (12.40) ab | (14.54) ab | (8.69) bc | (10.45) a | (11.87) a | (13.91) a |
| 7       | Chitosan /Ampelomyces quisqualis | 1.50 | 2.54 | 4.18 | 5.83 | 2.07 | 2.93 | 4.15 | 5.80 |
|         |                           | (7.01) abc | (9.14) a | (11.77) a | (13.94) a | (8.23) a | (8.81) a | (11.73) a | (13.90) a |
| 8       | Untreated Control         | 1.84      | 3.66   | 5.65   | 7.50   | 3.31      | 4.66   | 5.89   | 7.46   |
|         |                           | (7.78) d | (11.01) d | (13.73) d | (15.87) e | (10.45) c | (12.47) b | (14.04) b | (15.85) b |
|         | LSD (p=0.05)              | 0.57      | 0.68   | 0.71   | 0.92   | 1.10      | 1.84   | 1.63   | 1.47   |

Means with the same letter are not significantly different. Fig. in the parenthesis show angular transform values.
On 4th day, PLW values were more than 5% in all treatments, but the untreated control had the highest value of 15.85. PLW value in Chitosan / *Ampelomyces quisqualis* treatment was only 13.90. Chitosan / *Ampelomyces quisqualis* treatment registered better results among all treatments.

In conventionally managed vineyards, powdery mildew is usually controlled by regular application of fungicides. However, the health and environmental impact associated with the use of these products, the development of resistance to certain fungicides and the demand for residue-free grapes provide incentives for minimising reliance on chemicals in viticulture, pointing to a need for effective organic alternatives (Gubler *et al.*, 1996, Hofstein *et al.*, 1996, Savocchia *et al.*, 2004). Chitosan as a foliar treatment is a good option to control the growth, spread and development of many diseases involving viruses, bacteria, fungi and pests (Rabea *et al.*, 2003). It is nontoxic for humans and also has a low environmental impact (Li *et al.*, 1992, Shahidi *et al.*, 1999). Chitosan oligomers of different molecular weight and degree of acetylation induced an accumulation of phytoalexins in grapevine leaves, which reduced *B. cinerea* and *Plasmopara viticola* infections (Ben-Shalom and Fallik, 2003). The induction of systemic resistance in plants with natural compounds like chitosan, is a promising approach to disease control (Gozzo, 2003). Kamble *et al.*, (2019) reported that preventive sprays of GI (Gamma irradiated) chitosan reduced the powdery mildew intensity in pea.

In the present study, three chitosan formulations, solo and in alternation with *Ampelomyces quisqualis* were evaluated for their field efficacy in the control of powdery mildew of grapes. Results revealed that chitosan formulations effectively inhibited powdery mildew on grapes when compared to untreated control. Results of the present investigations on the efficacy of chitosan formulations had close resemblance with earlier investigations. Iriti *et al.*, (2011) evaluated the new chitosan formulation and reported the significant reduction in the PDI of powdery mildew particularly at the concentration of 0.1%, even under high disease pressure conditions. Chitosan provided the best grapevine downy mildew protection without affecting the quality of grape production (Romanazzi *et al.*, 2016). Earlier investigations recorded the efficacy of chitosan against powdery mildew (*Blumeria graminis f. sp.hordei*) in barley, as well as its effective antiviral activity in tobacco and bean crops (Iriti *et al.*, 2006, Faoro *et al.*, 2008, Iriti and Faoro 2008).

Combined application of BCAs with different plant elicitors to target different stages in the infection process, was one of the approaches to increase the reliability and level of activity of biological control treatments (Schmitt *et al.*, 2001). Where a single biological method does not give sufficient protection, such approach is essential (Elad *et al.*, 1996). In our studies, the formulations were found more effective when used in alternation with *Ampelomyces quisqualis*. Results of the present investigations are in tandem with earlier investigations indicating the effectiveness of chitosan with bio-control agents. Giotis *et al.*, (2012) used the combination of biological control agents (BCAs) *Ampelomyces quisqualis*, plant defence elicitors Milsana® VP 2002 (a plant extract of *Reynoutria sachalinensis*) and chitosan and reported that the combination had significantly reduced cucumber powdery mildew incidence as compared to untreated control. El-Mohamedy *et al.*, (2014) analysed *T. harzianum* and chitosan (0.5 and 1.0 g/l) as combined treatments for controlling *Fusarium* crown and root rot of tomato and reported that the combination had significantly reduced
**Fusarium** crown and root rot disease. The genus *Ampelomyces* is a potential biocontrol agent against the powdery mildew pathogens (Kanipriya *et al.*, 2019) and Kiss (2003) reported its commercial formulation in controlling the powdery mildew menace.

Biocides are effective in maintaining the physiological loss in weight (PLW) of grapes. Chlorine dioxide (ClO2) played an important role in reducing the PLW at 200 ppm concentration (Sharma *et al.*, 2017). In our study the physiological loss in weight (PLW) was low in all treated plots as compared to untreated control plot. Treatment chitosan / *Ampelomyces quisqualis* showed significantly lower physiological loss in weight (PLW) than untreated control. Chitosan coating improves the shelf life and postharvest quality of table grape (Suryawanshi, 2018). It is clear that, chitosan application played an important role in reducing the PLW. Hence, chitosan can be effectively used in powdery mildew management as well as improve the shelf life of berries, either alone or in alternation with *Ampelomyces quisqualis*.

Our study revealed that chitosan formulations have a potential to control the plant diseases. Though interesting theoretical and applied findings were gathered in recent years, more are needed to examine the mechanisms governing the mode of action of these compounds. In the case of antimicrobial mode of action, future work should aim at clarifying the molecular details of the underlying mechanisms and their relevance to the antimicrobial activity of chitosan. Therefore, future research should be directed towards understanding their molecular level details, which may provide insights into the unknown biochemical functions of chitosan formulations as well as help to accelerate their future and might assist in the goal of sustainable agriculture.

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