**Report of Research Trial**

**On**

**Evaluation of the bio-efficacy of Microshield against post-harvest and shelf life in grapes**

**(2019-2020)**

**Sponsored by**

**NBS Singapore Pte Ltd**

## Introduction

Post-harvest losses in grapes are important as quality and cost factors make the market more competitive. Post-harvest deterioration of grapes can be due to physical, physiological or pathological factors that may occur in the vineyard (pre-harvest) or after harvest. Loss during harvesting and post-harvest loss is a common phenomenon in grapes due to the perishable nature of the crop. The post-harvest loss in grapes has been recorded by various workers within the range of 8.23 to 16 per cent in the country. Control of the post-harvest losses will help reduce the losses for farmers and enhance the profitability of grape production by way of increased exports.

The major post-harvest fungal damages of grapes in Maharashtra, India during low-temperature storage, are caused by Alternia infections, which occur mainly at the pedicle end, and Cladosporium infections of berries caused by bruising and injuries inflicted during harvesting, handling or transport. At warmer temperatures, Aspergillus infections result in split or cracked berries; infections of Rhizopus stolonifer were observed in grapes which got wet due to pre-harvest rains or water from dew, spray, etc. Alterneria, Claudosporium, Aspergillus and Penicillium spp. They were found to be associated with individual berries, while Rhizopus stolonifer was associated with bunch rot. Botrytis rot is not observed in this region.

Although the post-harvest losses can be reduced if proper handling and care are taken during harvesting, transporting, cleaning, grading and packing to minimize the injury caused to the berry, complete elimination of the disease is not possible. Giving prophylactic or pre-harvest sprays of chemicals or biological help in reducing the inoculum of fungus on berries, and prompt refrigeration at 0 +_ $0.5^0$ C after harvesting helps in minimising the development of pathogens during transit and storage.

Microshield contains Trichoderma viride- 1% WP is an eco-friendly culture of microorganism containing Trichoderma viride spores effective against a wide variety of seed and soil-borne plant pathogenic fungi.

The Hyphae of Trichoderma wrap around the pathogen fungi and produce antibiotics and extracellular enzymes, which lyses the cell wall of these pathogens that damage them. The invading fungus eventually collapses and disintegrates.

1. Material and Methods

1.1. Applications of Microshield (Trichoderma viride 1.15% A.S.)
1.2. In vineyards

The experiment was conducted in the vineyard of table grape cv. Sonaka was located in Nashik in April 2019. The vineyard was maintained with a standard package of practices till 10th April 2019.

NBS Singapore supplied the test fungicide, Microshield (Trichoderma viride 1.15% A.S.) Two spray applications of fungicides were carried out. The first spray was given on 2nd April 2019 and the second spray was given on 7th April 2019. The water volume used for spray was calculated based on the requirement of 1000L/ha at the full canopy sprayer used for spray. Knapsack sprayer was used for spray.

Details of treatments are given in Table 1 (a). Dates of fruit pruning and fungicide sprays at the location are mentioned in Table 1 (b).

**Table 1 (a). Details of the treatments**

| Tr. No. | Treatment | Dose (ml/L) |
|---|---|---|
| T1 | Azoxystrobin 23 SC | 0.5 |
| T2 | Microshield | 2.5 |
| T3 | Microshield | 5.0 |
| T4 | Microshield | 7.5 |
| T5 | T1 followed by T2 | 0.5+2.5 |
| T6 | T1 followed by T3 | 0.5+5.0 |
| T7 | T1 followed by T4 | 0.5+7.5 |
| T8 | Control (Water spray) | - |

**Table 1 (b). Dates of pruning, harvesting and fungicide sprays**

**Date of pruning: 20/11/2018**

| Spray No. | Date of spray | Days after pruning |
|---|---|---|
| 1 | 02/04/2019 | 134 |
| 2 | 07/04/2019 | 139 |

**Date of Harvesting: 10/04/2019 (142 days)**

**2.2. Harvesting, grading, packing and storage of grapes**

Good quality grapes were harvested from each treatment. These grapes were graded and packed in 500 g capacity punnets (small light plastic containers for fruit storage) and 10 punnets were put in 5.0 kg cardboard boxes, the dimensions of which were designed for export of grapes. There were 10 replicates per treatment. Cardboard boxes were precooled and were kept at cold storage for 15 days at 0 deg C +- 1. After 15 days boxes were brought to room temperature (28-30 deg C ), for further observations.

**2.3. Observations on physiological loss in weight (PLW)**

Observations on the weight of bunches were recorded for all treatments at 24-hour intervals for the first 7 days at room temperature. The per cent loss of weight over the initial weight was calculated mathematically for grapes in each replicate

### 2.3.1. Observations on fallen berries and rotten berries

The observations were recorded on the 7th day after keeping the punnets at room temperature. The number of berries separated from rachis in each punnet was recorded as fallen berries.
The berries showed any symptom of rotting or growth of fungus or bacteria, numbers of such berries were recorded as rotten berries. The data was represented in terms of the percentage of fallen and rotten berries.

### 2.3.2. Observations of freshness ratings

The grapes in each of the ten punnets were critically observed on 18th April 2019 (7th day at room temperature) to give freshness ratings on 1-5 scale as detailed below:

| Rating | Description |
|---|---|
| 1. | More than 50% of the rachis turned black and more than 10% of berries showing shriveling or rotting. |
| 2. | About 50% of the rachis turned dark brown and 5 to 10% of berries showed shriveling or rotting. |
| 3. | About 25% of the rachis turned dark brown and few berries showed shriveling or rotting symptoms. |
| 4. | The rachis is green, and no shriveling or rotting of berries is present, but the main rachis at cut the end is dried and turned to dark brown. |
| 5. | Bunches are considered fresh as rachis is green and no shriveling or rotting of berries present. |

The average of 10 punnets from each treatment has been presented in tables.

### 2.4. Statistical Analysis

The post-harvest shelf-life data was analyzed statistically following a completely randomized design (CRD) using a Statistical Analysis System (SAS software 9.3) Means were compared using Tukey's Studentized Range (HSD) Test.

## 3. Results

### 3.1. PLW (Physiological loss in weight)

PLW of grapes was estimated after keeping them on the shelf at room temperature and data is presented in Table 2. The PLW of 5% is generally accepted as the threshold value for the grape bunches to be considered fresh. Hence data is presented till the 5th day on the shelf, when the PLW increased by more than 5% and hence was considered unfit.

PLW of grapes in all treatments treated with Microshield (Trichoderma viride 1.15% A.S.) did not show a significant difference as compared to the untreated control.

### 3.2. Fallen berries

None of the treatments showed significant differences on the reduction of fallen berries which was treated with Microshield (Trichoderma viride 1.15% A.S.) as compared to the untreated control (Table 3).

### 3.3. Rotten berries and Freshness ratings

All the treatments significantly reduced rotten berries and significantly high freshness ratings over untreated control. Microshield @ 5 g/L or 7.5 g/L water alone or in combination with azoxystrobin 23 SC @ 0.5 g/L water reduced rotten berries and increased freshness rating better than other treatments and untreated control (T6 and T7) (Table 3).

**Table 2. PLW of Grapes at room temperature**

| Sr.no. | Treatments | Physiological loss in weight (PLW) | | | | | |
|---|---|---|---|---|---|---|---|
| | | a.i.(g) | 1 day | 2 day | 3 day | 4 day | 5 day |
| 1 | Azoxystrobin 23 SC | 0.5 | 2.13a | 3.48b | 8.83a | 13.04ba | 15.37a |
| 2 | Microshield | 2.5 | 2.20a | 4.12a | 8.71a | 12.22bac | 14.06ba |
| 3 | Microshield | 5.0 | 1.90a | 3.55ba | 7.32b | 10.90bc | 13.06bc |
| 4 | Microshield | 7.5 | 1.70a | 3.39b | 6.94b | 10.01c | 12.01c |
| 5 | T1 followed by T2 | 0.5+2.5 | 2.21a | 3.76ba | 7.36b | 10.51c | 12.57bc |
| 6 | T1 followed by T3 | 0.5+5.0 | 1.87a | 3.89ba | 7.89ba | 11.25bac | 13.68b |
| 7 | T1 followed by T4 | 0.5+7.5 | 1.89a | 3.45b | 7.28b | 13.43a | 13.28bc |
| 8 | Control (water spray) | - | 2.16a | 3.89ba | 7.77ba | 11.11bac | 13.28bc |
| | CD (p=0.05) | | **0.66** | **0.60** | **1.30** | **2.48** | **1.66** |

**Table 3. Percentage of fallen berries, rotten berries and freshness rating**

| Sr.no. | Treatment details | a.i.(g) | Fallen Berries 7$^{th}$ day | Rotten berry (%)7$^{th}$ day | Freshness rating 7$^{th}$ day |
|---|---|---|---|---|---|
| 1 | Azoxystrobin 23 SC | 0.5 | 0.00 | 4.0 | 1.30 |
| 2 | Microshield | 2.5 | 0.00 | 3.30 | 1.40 |
| 3 | Microshield | 5.0 | 0.60 | 1.70 | 2.30 |
| 4 | Microshield | 7.5 | 0.00 | 1.10 | 2.50 |
| 5 | T1 followed by T2 | 0.5+2.5 | 0.10 | 3.20 | 1.50 |
| 6 | T1 followed by T3 | 0.5+5.0 | 0.00 | 1.20 | 2.40 |
| 7 | T1 followed by T4 | 0.5+7.5 | 0.30 | 0.70 | 2.70 |
| 8 | control (water spray) | - | 0.00 | 7.40 | 1.00 |
| | CD (p=0.05) | | **0.72** | **1.28** | **0.66** |

## 4. Conclusion

The pre-harvest spray of Microshield (Trichoderma viride 1.15% A.S.) @ 5 g/L or 7.5 g/L water or in combination with a spray of azoxsystrobin 23 SC @ 0.5 g/L water showed improvement in shelf life by reducing rotten berries and improving overall freshness ratings.