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Research Article

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EVALUATION OF FLAXSEED OIL AND TRICHODERMA SPECIES AGAINST GREY MOLD DISEASE OF GRAPES

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ABSTRACT

Grapes (*Vitis vinifera* L.) are included in vital crops in the world with respect to its nutritious and economic value. Botrytis grey mold, also called noble rot, harms grapes and wine quality caused by *Botrytis cinerea*. Other than chemicals *Trichoderma* species considered as important bio control agents for management of post-harvest diseases. The current study was designed to evaluate *Trichoderma* species for their Micoparasitic ability using a modification of a pre-colonized plate method. *In-vitro* efficacy of flaxseed oil against the *Botrytis cinerea* of grapes were determined using disc diffusion technique with different oil concentrations 0.2%, 0.6%, and 0.8%. *In-vivo* efficacy of most effective formulated and non-formulated *Trichoderma* concentrations was determined by applying on grape bunches against *Botrytis cinerea*. *Trichoderma Harzianum* is more effective and showed 67.5% growth inhibition than *Trichoderma Viridae* having 64% growth inhibition while flaxseed oil with 0.2%, o.6% and 0.8% concentrations showed 30.19%, 33.32% and 34.89% growth inhibition against *botrytis cinerea* respectively. In water-based *Trichoderma* concentrations (1×10^8) showed maximum growth inhibition after 6 days that was 76.46%. However, it was observed that the percent disease severity index of the grape fruits in oil-based formulation showed 16 %, 20 %, and 18% after the 9 days of incubation whereas the water-based *Trichoderma* formulations showed 20%, 22%, 24% respectively as compared with control. *Trichoderma* species can be considered as bio-control agent while emulsion oil can be used for better shelf life.

Keywords: *Botrytis cineria*; Grapes grey mold; *Trichoderma Harzianum*; *Trichoderma viridae*, oil

INTRODUCTION

Grapes (*Vitis vinifera* L.) are included in vital crops in the world with respect to their nutritional and economic value. All over the world, grapes are cultivated for consumption, wine, juices and raisins. Grapes are rich in vitamin A, calcium, iron, and are good for heart disease prevention since they boost human immunity (Ali et al., 2010). Carocho & Ferreira, (2013) the grape's anthocyanin component has several positive impacts on the brain and central nervous system. It has anti-oxidant components that help reduce the risk of liver disorders, cancer, and heart attacks.

Grapes has almost sixty species of family *Vitaceae* but commonly three species of grapes known to us i.e. The European grapes (*Vitis vinifera* L.), The American grape (*Vitis labrusca* L.), and Muscadine grapes (*Vitis rotundifolia* M). The production in Millions of tons. According to reports from Pakistan, post-harvest losses of grapes ranged from 16 to 22 percent as a result of fungi, improper packaging, inadequate transportation, and bad marketing strategies (Aujla et al., 2011). Post-harvest infections are a key cause of post-harvest losses and shorten the period of storage among all these factors generating a reduction in grape losses.

During the growing season as well as after the harvesting of grapes, there are several diseases occur on grapes which are mainly caused by fungus, bacteria, nematodes and viruses. In the post-harvest diseases, Botrytis bunch rot or grey mold which is also known as noble rot of grapes and is affected by the fungus *Botrytis cinerea* is unusual disease causes significant losses in grapes yield and also decrease the wine quality throughout the world. *B. cinerea* is the second most damaging pathogenic fungi after *Magnoparthae oryzae* (T.T. Herbert, M.E. Barr) which causes the rice blast disease. *B. cinerea* also infects more than 30 species of plants and causes a number of diseases such as rotting.

Furthermore, for best management, a prolonged exposure to free water is frequently necessary. Therefore, there has been a lot of interest in creating these microbe formulations that improve microbial survivability in the lack of free moisture. One of these formulations uses an emulsion of water and oil (invert). This kind of emulsion has demonstrated potential as a water supply to promote fungal infection and as a carrier for weed infecting fungi (mycoherbicides)(Batta, 1999). These fungi can also use for the management of foliar diseases like powdery mildew, gray mold and also for the management of post-harvest decaying fungi (Sawant, 2014).

Botanicals such as oils and extracts have been effectively used by researchers from throughout the world to reduce post-harvest rotting fungus. (Reverchon, 1997). Control of grey mold caused by *Botrytis cinerea* which is one of the most destructive postharvest decays causing pathogen of grapes. Severe losses have been reported due to *botrytis cinerea.*

Biological control of grey mold of grapes disease is practicing in developed and under developing countries by using of various types of scientific approaches like using of several biological agents like (Bacteria, fungus and nematodes etc.) or by using of plant extracts. Živković et al. (2010). *Trichoderma spp*. is also the most exploited and studied class of biological control agent. The application of *Trichoderma spp*.is mainly used for the soil borne pathogens to control the soil borne diseases or root diseases. But these fungi can also use for the management of foliar diseases like powdery mildew, gray mold and also for the management of post-harvest decaying fungi (Sawant, 2014). A variety of *Trichoderma* based formulations are commercially available for crop production. (Gary E Harman, 2000).

According to studies, flax is a plant that has been a reliable component of farming for hundreds of years and that can be used in all parts of the plant. Plant "lignans" are another effective healing component that can be found in the flax seed's fibrous shell. The immune system is stimulated by lignans, which also have antibacterial, antifungal, and antiviral properties. When several extracts of flaxseed powder were tested for their ability to combat bacteria and fungi, the results showed that the seeds' raw extracts did indeed possess these properties (Narender et al., 2016).

Use of *Trichoderma* specie as bio control agent against *Botrytis cinerea* causing postharvest decay of grapes which would be the alternative to the synthetic chemicals and an ecofriendly approach which is safer for the consumers health with no residual effects. With the potential and benefits indicated above in mind, it is anticipated that the focus of the current research will be on identifying grape post-harvest fungal diseases in order to facilitate the implementation of improved management practices.

The study is focusing the advances in the development of formulation of *Trichoderma* to enhance its bio-control efficacy against post-harvest pathogens. A bio-control preparation of *Trichoderma* as *Trichoderma* emulsion using mineral oil for better shelf life, cost effective and environmentally friendly approach also, the objective of present research was to formulate the *Trichoderma* using a stable and invert emulsion and check its biological efficacy against *Botrytis cinerea* of grapes at post-harvest stage.

MATEARIAL AND METHODS

The study comprised bio-management of post-harvest fungal pathogen causing the grey mold disease on grape fruit. Infected fruit samples of grapes (sunder khani and taffy) were collected from local market for isolation and purification of fungal pathogen. Cultural (colony color and texture) and morphological (spore, size, shape, and septation) characteristics of the isolated fungus were identified under Biological Light Microscope (Nikon YS100, USA).

Pathogenicity test: Pathogenicity was done on fresh grape bunches after surface sterilization. The grapes were punctured with a sterile needle upto 4-5 mm in diameter. Subsequently, a small disc containing *Botrytis cinerea* isolate from a 7-day-old culture was placed onto the injured grape bunches. Grapes inoculated with distilled water served as a negative control. Treatments were replicated thrice following incubation at 25 ℃ for four days in a sterile container (Senthil, Prabakar, Rajendran, & Karthikeyan, 2011).

***In-vitro* Screening of *Trichoderma* Species:** Two strains of *Trichoderma* were assessed for their ability to act as mycoparasites using a modified pre-colonized plate method. The fungal pathogen, *Botrytis cinerea*, was inoculated on PDA media. On the same PDA plate, a 0.5 x 2.5 cm segment of *Trichoderma* isolate was placed on the opposite side from the pathogen followed by incubation at 25°C for seven days. Percentage inhibition (PI) was evaluated by the following formula;

$$\text{PI} = \frac{C - T}{C} \times 100$$

PI= % inhibition
C= Control
T= Treatment

In-vitro Screening of Flaxseed Oil against *Botrytis cinerea:* The efficacy of flaxseed oil was determined against the *Botrytis cinerea* using disc diffusion Technique described by (Wang, 2002) using different oil concentrations (0.2%, 0.6%, and 0.8%). The oil disc was inoculated near the edge of Petri plate containing PDA media and 5cm disc of pathogen inoculum was placed at the opposite edge followed by incubation at 25±2°C for seven days. Experiment was carried out in a completely randomized design (CRD) and after seven days the colony diameter of fungal growth was measured, and the growth inhibition percent of treatments were compared to the control treatments using the formula;

$$\mathrm{MGI}\ (\%) = \frac{(\mathrm{Dc} - \mathrm{Dt})}{Dc} \times 100$$

Where, MGI = Mycelial Growth Inhibition (%)
Dc = Fungal Growth of Pathogen in control
Dt = Fungal growth of Pathogen in treatment

In-vitro* efficacy of formulated and non-formulated forms of *T. harzianum* against *B. cinerea: There was total six treatments with different concentrations, in which three treatments were water based and three treatments were oil-based *Trichoderma* emulsion with most effective concentration of oil. There were both +ve and –ve control for each treatment. Each treatment has 3 replications. 6 types of treatments with different concentrations of non-formulated *Trichoderma* and formulated *Trichoderma* were applied against *Botrytis cinerea*. three treatments were oil-based formulation and three were water based non formulated treatments and in –ve control we use water. In Water based non formulated treatments of conidial suspension were prepared in sterile distilled water. In other three oil-based formulated treatments Conidia mixing with combination of oil for stable formulation. We were use twen80 in oil-based formulation for stable emulsion. two readings were taken after 6 and 9 days. the growth inhibition percent of treatments were compared to the control treatments and calculated by the formula (Amini et al., 2012).

$$\mathrm{MGI}\ (\%) = \frac{(\mathrm{Dc} - \mathrm{Dt})}{Dc} \times 100$$

Where, MGI = Mycelial Growth Inhibition (%)
Dc = Fungal Growth of Pathogen in control
Dt = Fungal growth of Pathogen in treatment

RESULTS AND DISCUSSION

A total of five pathogenic isolates were identified as *Botrytis cinerea* based on their cultural and morphological traits. Conidia observed were obovoid to ellipsoid in shape having light brown to pale brown color. The largest size (L x W) observed was 15 x 8mm while minimum size was 9 x 2.3mm (Figure 1 A-C). Pathogenicity test indicated that all the five isolates were pathogenic, while Br-1 isolate was identified highly virulent showed more than 60% fruit rotting (Figure 2).

Figure 1: Cultural and morphological characteristics of botrytis cinerea (A) first view of the pathogen's growth in Petri plates, (B) top side image of the pathogen's growth in Petri plates over eight days and (C) microscopic view of the 1conidiophore with conidia bearing structures

Figure 2: Rotting of fruit after inoculation confirming pathogenicity test.

***In-vitro* Screening of *Trichoderma* Species:** The *Trichoderma harzianum* showed 67.83 percent growth inhibition while *Trichoderma viridae* showed 63.91 percent growth inhibition using dual culture technique (Figure 3 and 4).

In-vitro Screening of Flaxseed Oil against *Botrytis: cinerea:* In 0.2% concentrations of oil showed 30.19% growth inhibition while 0.6% and 0.8% flaxseed oil concentrations showed 33.32% and 34.89% growth inhibition respectively against *Botrytis cinerea*. It was observed that 0.8% flaxseed oil concentration showed maximum results against pathogenic isolates (Figure 5 and 6). The mechanisms used by *Trichoderma* spp. to antagonize fungal pathogens are competition, antibiosis, colonization and immediate mycoparasitism (Gary E Harman, 2011). This antagonistic potential provides the foundation for effective biocontrol applications against a wide range of plant pathogens (Lorito, Woo, Harman, & Monte, 2010). Singh, Yadav, Verma, and others, (2017) explained that post-harvest pathogen infection on fruits happens prior to harvest, stays dormant, and manifests itself mostly during ripening, storage, and transport. Dean et al. (2012) stated that critical plant diseases, such as *Botrytis cinerea* Pers. Fr., can infect plants at any stage of growth, including petioles, leaves, flowers, and fruits. The fungus doesn't show any symptoms and stays dormant until conditions are favorable for it to flourish.

Figure.3: Dual Culture of *Trichoderma harzianum* and *Trichoderma viridae.*

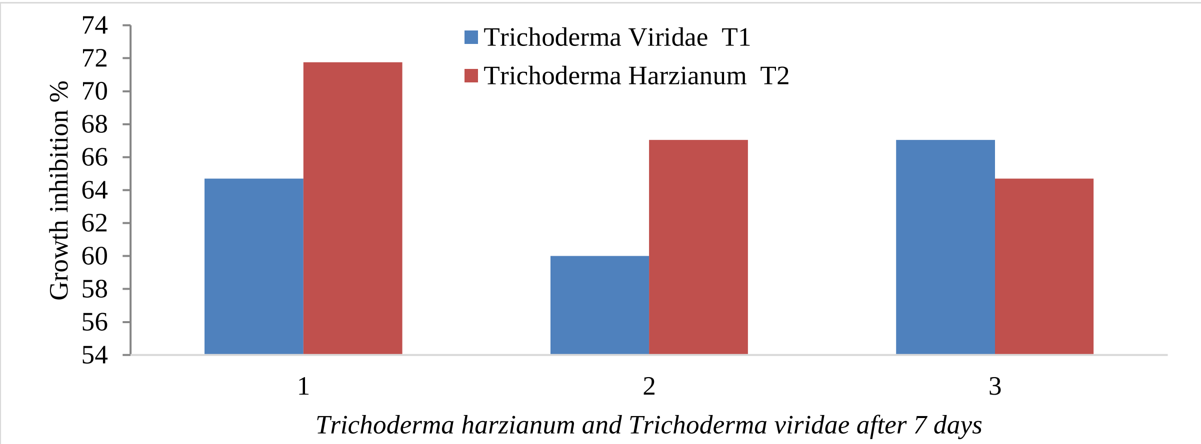

Figure.4: Percent growth inhibition of *Trichoderma* species after 7 days of inoculation.

Figure. 5: Disc diffusion for oil and *Botrytis cinerea*

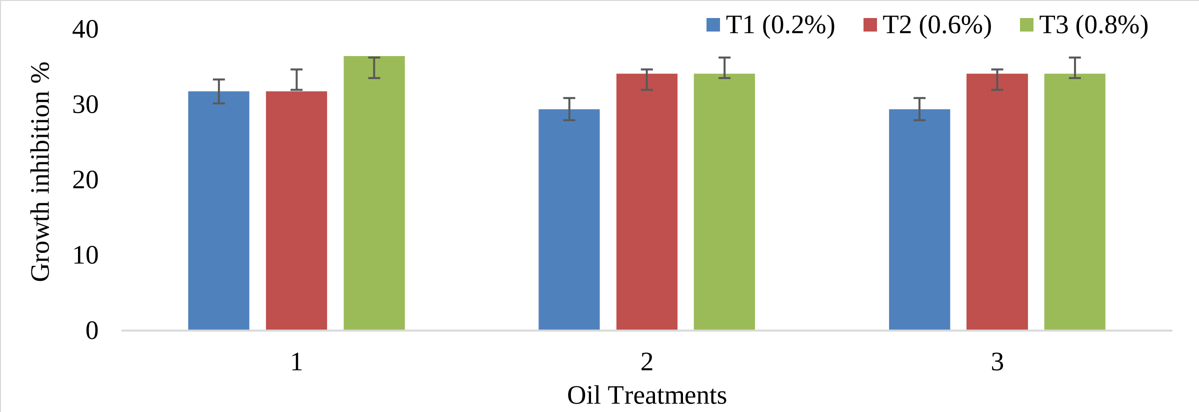

Figure 6: Growth inhibition percentage using flaxseed oil concentrations.

In-vitro* efficacy of formulated and non-formulated forms of *T. harzianum* against *B. cinerea: In water-based *Trichoderma* concentrations 1×10⁶ showed 65.48, while 10⁸ showed 76.46 growth inhibitions after 6 days. After 9 days water-based *Trichoderma* concentrations showed 63.11, and 72.54% growth inhibition respectively**.** So, 1×10⁸ showed most effective results against *Botrytis cinerea*. on the other hand, formulated *Trichoderma* emulsion (10^6, 10^7, 10^8 and 0.8% oil) showed 70.19, 72.52 and 79.60% growth inhibition respectively after 6 days while after 9 days showed 67.83, 69.41 and 77.25% inhibition respectively (Figure 7 and 8). The results are similar with the previous studies described by Ahmed et al., 2019 and Harman, 2000.

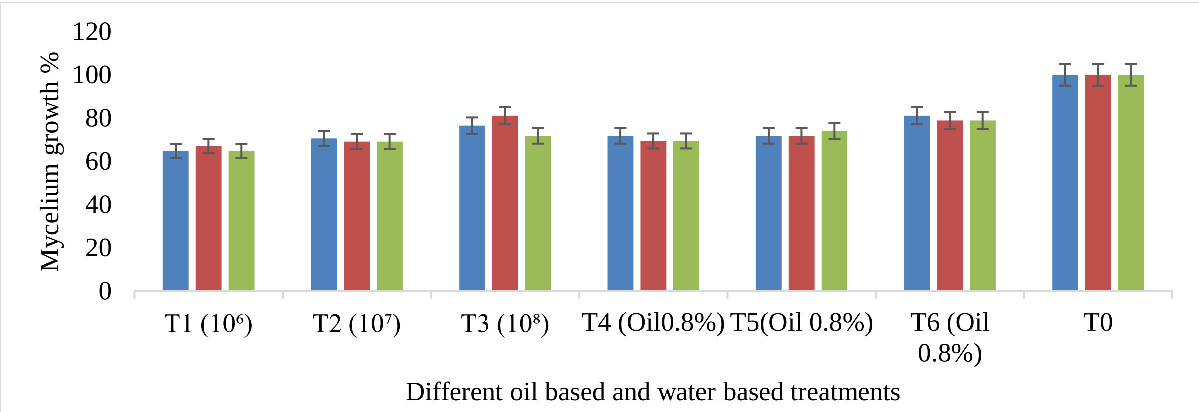

Figure. 7: Graphical Representation of Different Formulations Results after Six Days.

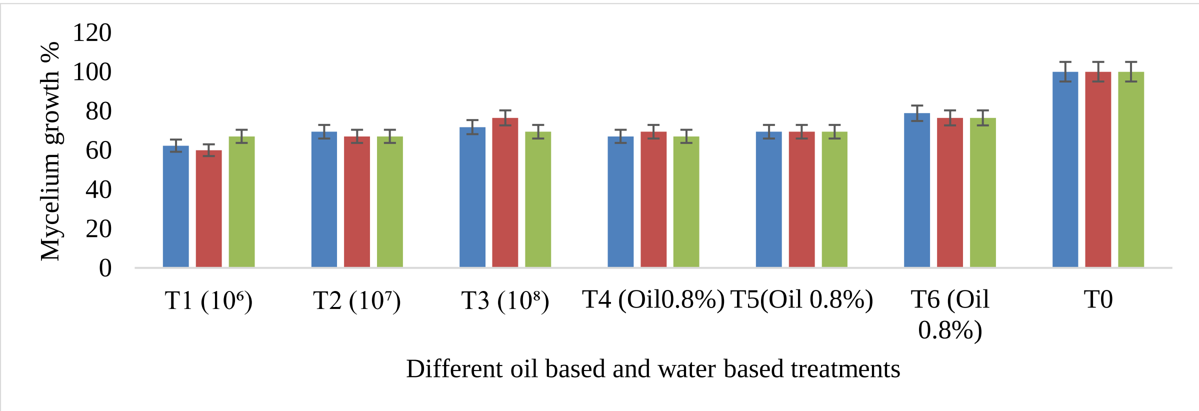

Figure 8: Graphical Representation of Different Formulations Results after Nine Days.

CONCLUSION

All formulated and non-formulated Trichoderma with different concentrations tested against botrytis cinerea to check mycelial growth inhibition percentage, and it was shown that while all concentrations showed antifungal behavior, the most effective treatment against the pathogen was a Trichoderma emulsion containing 0.8 percent oil. The concentration of 0.8% oil at 10^8, however, produced the most significant results.

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