

## EVALUATION OF DIFFERENT FUNGICIDES, BOTANICAL EXTRACTS AND BIOCONTROL AGENTS AGAINST *PENICILLIUM EXPANSUM* THE CAUSAL AGENT OF BLUE MOLD OF APPLE

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### ABSTRACT

Efficacy of various fungicides, their botanical extracts and bio-control agents against *Penicillium expansum*, the agent which cause blue mold of apple. Incidence of disease was recorded in Hyderabad and Tandojam. Various fungicides like Topsin-M, Melody Duo, Antracol, Cabriotop and Alliette at the different doses i.e. 100, 200 and 300 ppm, their botanical extracts like Ginger, Neem, Eucalyptus, Garlic and Onion, it was carried out at different doses i.e. 5, 10 and 15% by food poisoning method, their Pathogenicity test which was also performed to see the severity of disease in apples. The results showed that maximum disease incidence 53.45% was observed from Hyderabad and 46.55% was observed from Tandojam. It was observed that the disc method of inoculation showed a higher percentage (93.44%) as compared to the injection method of inoculation 65.55%. The growth of minimum linear colony of *Penicillium expansum* were observed for Topsin-M as 39.33, 32.00 and 25.00 mm respectively followed by Antracol 42.66, 35.00 and 30, Melody Duo 40.66, 35.66 and 30.00, Alliete 44.66, 36.00 and 31.00 and Cabriotop 46.33, 40.00 and 33.66. The growth of linear colony *Penicillium expansum* was observed as 41.00, 35.00 and 27.66 mm for Ginger respectively followed by Neem 45.00, 38.00 and 33.66, Eucalyptus 48.00, 41.00 and 35.33, Garlic 52.33, 45.33 and 39.66) and Onion 61.66, 52.00 and 46.00. The linear colony growth of *Penicillium expansum* was minimum to observed for *Guignardia* sp. 29.66 mm, followed by *Chaetomium* sp. 35.33mm, *Hypoxyylon* sp. 35.66mm, *Fusarium* sp. 40.66mm, *Trichoderma* sp. 47.66mm.

**Keywords:** Apple, Blue Mold, Fungicides, Botanical Extracts, Biocontrol, *Penicillium Expansum*

### INTRODUCTION

Apple is one of the most important fruit in fruit crops, which is highly accessible and widely consumed in the daily life of the human. Apple fruit is scientifically known as (*Malus domestica*) belongs to the family rosary. It is one of the widely cultivated fruit all over the world. Most of the apple varieties are cross-pollinated. When harvested they are usually round in shape, 2-4 inches (5-10 cm) in diameter and having green, red or yellow shades. Their size, shape and acidity depending on the variety used. It is the second most cultivated fruit in Balochistan after dates. Golden Delicious (Shin Kulu), Red Delicious (Tor Kulu), Kaja, Mushhadi, Amri and Kashmiri are commonly grown in Balochistan (USDA, 2013).

Apple has been cultivated in tropical and subtropical climatic conditions of Kenya, Zimbabwe, Brazil, Mexico, India where the altitudes were high (Ashebir *et al.*, 2010; Wamocho and Ombwara, 2001).

Apple contains numerous phytochemicals which are beneficial for health and effective against a numerous health risk such as cancer, asthma, cardiovascular and Alzheimer diseases (Hyson, 2011). *M. sieversii* species of apple has been grown in Kazakhstan and Kyrgyzstan (Pereira-Lorenzo *et al.*, 2009). The species hybridized with *M. sieboldii*

and *M. baccata* in East and *M. sylvestris* and *M. turkmenorum* in West. In Europe and the Mediterranean region, well-established apple cultivar then introduced by Romans (Juniper *et al.*, 1999).

Production, quality and quantity of apple is diminished by the attack of fungi during the maturity of the plant in the field condition. Among them, blue mold decay caused by *Penicillium* sp. leads to significant economic losses during storage that also impacts on fruit destined for processing by its productivity of the carcinogenic mycotoxin patulin (Barkai-Golan, 2008). *P. expansum* infect fruit by wounds in bruises or stem punctures at harvest and post-harvest handling and enter the fruit through lenticels, stem ends and calyx end (Rosenberger *et al.*, 2006). When they enter inside the fruit the soft lesions and light-colored symptoms appear on the fruit. *P. expansum* virulence due to maceration of tissue plays a significant role (Jurick *et al.*, 2010). The fungus grows on the lesion surface and surrounds the tissue then cause wound infection in healthy fruit by damaging the decayed portion (Sommer *et al.*, 2002).

*Penicillium expansum* damage the fruit in countries where fungicide usage prohibited after harvest (Jonsson *et al.*, 2010). Significantly increased fungal diseases in Europe during the last dec-

ade's most likely with global warming was being one of major cause (Weber, 2009). Although blue mold in apple varieties are inherently sensitive and thus greater than 10% of fruit harvested was lost during storage (Tahir, 2014).

*Penicillium* species was first described by Link (1809) over 200 years ago. The morphological features of *Penicillium* species have been seeking towards the more holistic approach within the genus (Frisvad and Samson, 2004; Pitt and Hocking, 2009). Keeping in view the significance of the disease, therefore this study is designed for the evaluation of various fungicides, botanical extracts and biocontrol agents against *Penicillium expansum* the causal agent of blue mold of apple.

## MATERIALS AND METHODS

### Sampling and Survey of apple infected fruits:

To visited various fruit markets at Tandojam and Hyderabad. Collected the infected specimens that were showing the symptoms of blue mould of apple. And then brought these specimens to the mycological laboratory at Plant Pathology Department, Faculty of Crop Protection, Sindh Agriculture University Tandojam, for and identification and isolation of the causal agent.

The diseases Incidence was obtained by applying the following formula.

$$\text{Incidence \%} = \frac{\text{Total number of plant}}{\text{Diseased plants}} \times 100$$

### Identification and Isolation of the causal fungus:

Fruits of apple infected with the disease were collected from different fields of Tandojam and Hyderabad. These were processed in Fungal Molecular Biology Lab, Plant Pathology Department, Sindh Agriculture University Tandojam. For the isolation of the pure culture of the fungal pathogen, a portion of fruit containing typical blue mold symptoms was sliced into 3-5 mm long pieces. These pieces were surface-sterilized for 3 minutes in 1% NaOCl solution followed by rinsing with three consecutive changes of sterilized distilled water and was placed on potato dextrose agar medium in Petri plates. The plates were incubated at  $26 \pm 2$  °C in incubator for mycelial growth. Plates were incubated at  $28 \pm 2$  °C in an incubator for mycelial growth. The growth of fungus and their colony was recognized on the basis of their morphological characteristics as reported by Ellis (2013) and Frank (2005). The frequency of isolated fungi and their information from different plant parts was calculating through this formula:

$$\text{Frequency \%} = \frac{\text{Number of pieces colonized by the fungus}}{\text{Total no of pieces cultured}} \times 100$$

**Cultural purity of the causal fungus:** Most frequently isolated fungal pathogen was purified, and mass cultured on potato dextrose agar (PDA)

media. Test tubes containing a pure culture of the isolated fungal pathogen was preserved in Fungal Molecular Biology Lab, SAU.

**Pathogenicity test:** There are two methods were used in this study, injection and disc method. 8 fresh and healthy apples were chosen. In the disc method, 5mm diameter agar disk of test fungus was cut from 8-10 days old culture plate by using sterile cork borer and placed it in the deep centre of the apples. While an injection method made the suspension of *Penicillium expansum* then inoculate the solution into the healthy apples through injection. Specimens were wrapped then kept these specimens at the lab. The test was observed daily for 10-15 days. The same fungus was re-isolate and confirmed.

**Efficacy of different fungicides:** In-vitro studies furthermore selective chemical fungicides viz., Melody-Duo, Topsin-M, Aliete, Antracol, and Cabrio-Top fungicides were evaluated against the pathogen caused by food poisoning method. For this purpose, 3 different chemical concentrations (100, 200, 300 ppm) were incorporated in the PDA medium before the poring and one medium excluding fungicide kept as control. After medium solidifying, agar disk (5mm) dia of test fungus cut from old culture (5-8) by cork borer and then poured in PDA plate center and incubated at 25°C. The radial colony growth of test fungus recorded by drawing two perpendicular lines on the back of the Petri plates crossed each other in the center of the plate. The data on colony growth was recorded along with these lines in millimeter after every 24 hours until the plates were filled in any treatment Followed by Mengal *et al.*, (2019)

**To check the antifungal potential of botanical extracts:** The efficacy of various botanical extracts e.g. Ginger, Neem, Eucalyptus, Garlic and Onion were used at different doses i.e. 05%, 10% and 15% by using food poisoning method. The experiment was designed as CRD with three replications, data was recorded and analyzed by using Statistics 8.1.

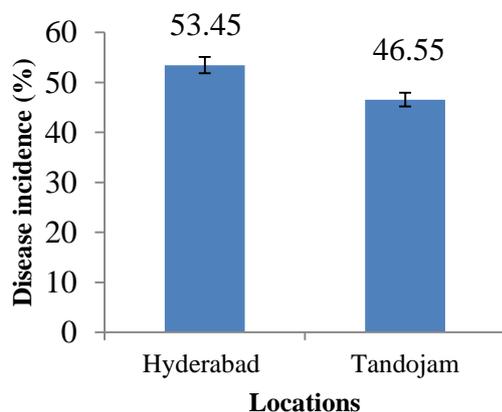
For this purpose, three different botanical extract concentrations (5, 10, 15%) light-coloured in the PDA medium before the poring. one medium excluding fungicide kept as control. After medium solidifying, agar disk (5mm) dia of test fungus cut from old culture (8-10) by cork borer and then poured in PDA plate center and incubated at 25°C. The radial colony growth of the test fungus was recorded by drawing of two perpendicular lines on the back of the Petri plates crossed each other in the centre of the plate. The data on colony growth was recorded along with these lines in millimetre after every 24 hours until the dish or plate was filled in any treatment Followd by Gadhi *et al.*, (2018).

**The efficiency of biocontrol agents:** Biocontrol agents like *Trichoderma victoria* and other biocontrol agents viz., *Fusarium* sp., *Chaetomium* sp. and *Hypoxylon* sp. were evaluated against *Penicillium expansum* causing blue mould of apples. For this purpose, dual culture technique was used Followed by Jatoi et al., (2016)

## RESULTS

This study was carried out to study the *in-vitro* efficiency of different fungicides, their botanical extracts and bio-control agents against *Penicillium expansum*, the causal agent of blue mold of apple. The incidence of disease was also recorded in Hyderabad and Tandojam. The efficacy of different fungicides like Topsin-M, Melody Duo, Antracol, Cabriotop and Allietteat different doses i.e. 100, 200 and 300 ppm, botanical extracts like Ginger, Neem, Eucalyptus, Garlic and Onion was carried out at different doses i.e. 5, 10 and 15% by food poisoning method to find out the effective and sustainable extracts for the growth inhibition of the fungus. Bio-control agents such as *Trichoderma* sp., *Hypoxylon* sp., *Fusarium* sp., *Chaetomium* sp. and *Guignardia* sp., were evaluated. The pathogenic test was also performed to see the severity of disease in apples.

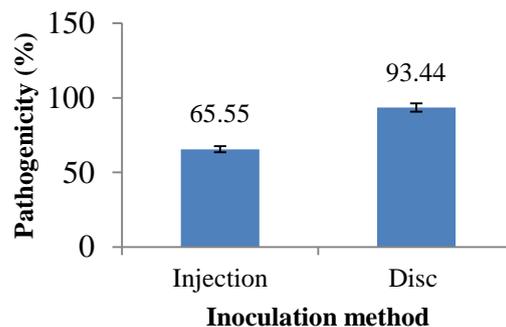
**Disease incidence (%):** Disease incidence in two locations such as Hyderabad and Tandojam was checked and presented in Figure-1. The data showed that higher disease incidence 53.45% was observed from Hyderabad and lower disease incidence (46.55%) was observed from Tandojam.



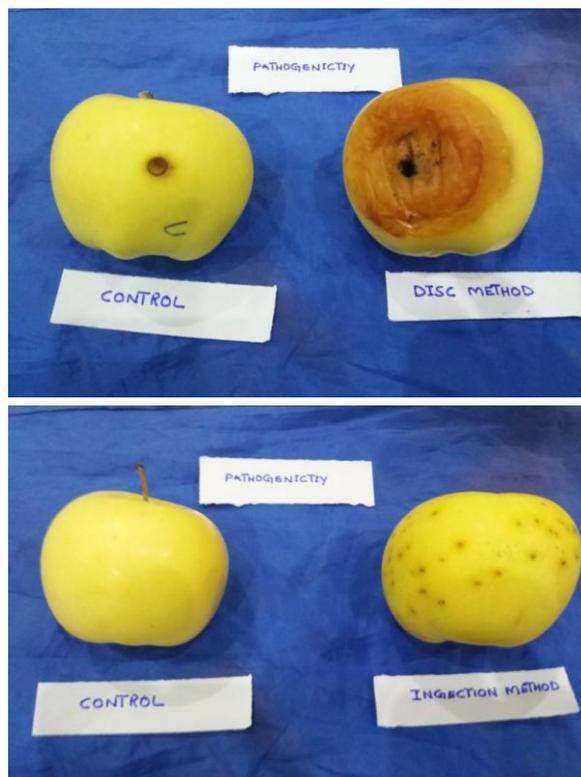
**Figure 1 :** The incidence (%) of blue mold of apple at different locations.

**Pathogenicity through injection and disc inoculation methods:** Results regarding the pathogenicity through injection and disc inoculation methods are presented in Fig-2 and 3. The data shows that 65.55 and 93.44 disease severity of *Penicillium expansum* in the apple was observed by injection and disc method of inoculation. On the basis of per-

centage, it was observed that disc method of inoculation showed a higher percentage 93.44% as compared to the injection method of inoculation 65.55%.



**Figure 2:** Pathogenicity through injection and disc inoculation methods



**Figure 3:** Pathogenicity through injection and disc inoculation methods

**Effect of different fungicides on the linear colony growth of *Penicillium expansum*:** The results presenting in Fig-4 and 5 showed that minimum linear colony growth of *Penicillium expansum* was observed as 39.33, 32.00 and 25.00 mm for Topsin-M at the doses of 100, 200 and 300 ppm, respectively followed by Melody duo 40.66, 35.66 and 30.00mm, Antracol 42.66, 35.00 and 30 mm, Alliette 44.66, 36.00 and 31.00mm and Cabriotop 46.33, 40.00 and 33.66 mm at the doses of 100, 200 and 300 ppm, respectively. The higher colony growth 90mm was observed under control. Statistical data revealed that there was a significant difference

among the fungicides at a different level of concentration for the linear colony growth of fungus.

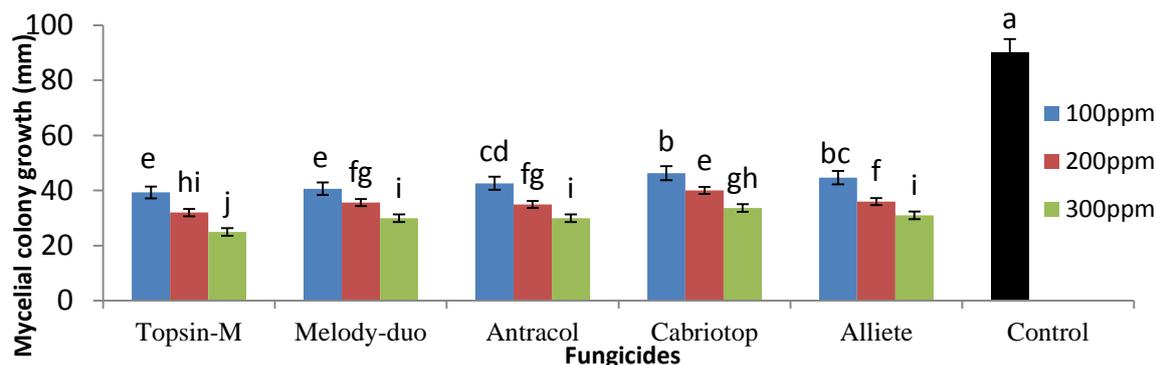


Figure 4: Effect of different fungicides on the linear colony growth of *Penicillium expansum* under *in-vitro* conditions.

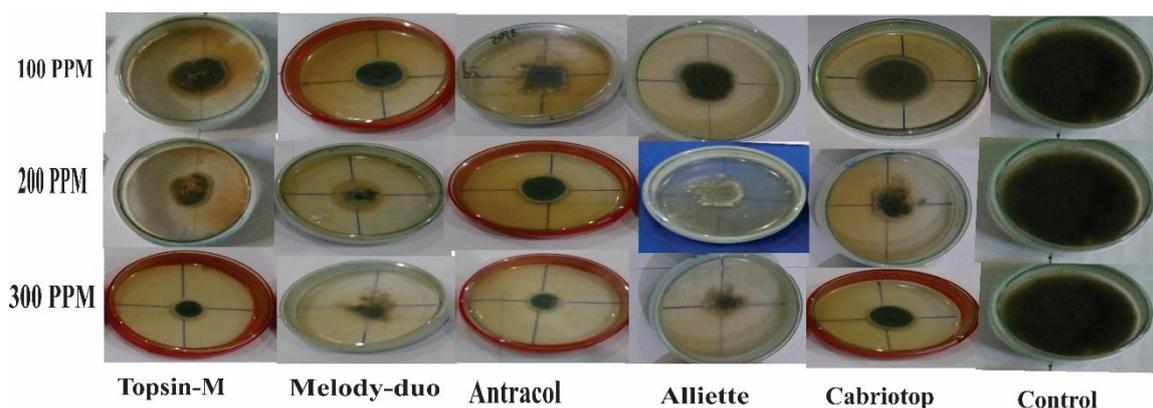


Figure 5: Linear colony growth of *Penicillium expansum* as influenced by fungicides under *in-vitro* conditions.

**Effect of different botanical extracts on the linear colony growth of *Penicillium expansum*:** The results presenting in Fig-6 and 7 showed that minimum linear colony growth of *Penicillium expansum* was observed as 41.00, 35.00 and 27.66 mm for Ginger at the doses of 5, 10 and 15%, respectively followed by Neem 45.00, 38.00 and 33.66 mm, Eucalyptus 48.00, 41.00 and 35.33 mm, Garlic 52.33, 45.33 and 39.66 mm and Onion 61.66, 52.00 and 46.00 mm at the doses of 5, 10 and 15%, respec-

tively. The maximum linear colony growth 90mm was observed under control. The minimum linear colony growth of *Penicillium expansum* was observed at 15% for Ginger followed by Neem, Eucalyptus, Garlic, and Onion, respectively. Statistical analysis of the data revealed that there was a significant difference among the botanical extracts at a different level of concentration for the linear colony growth of fungus.

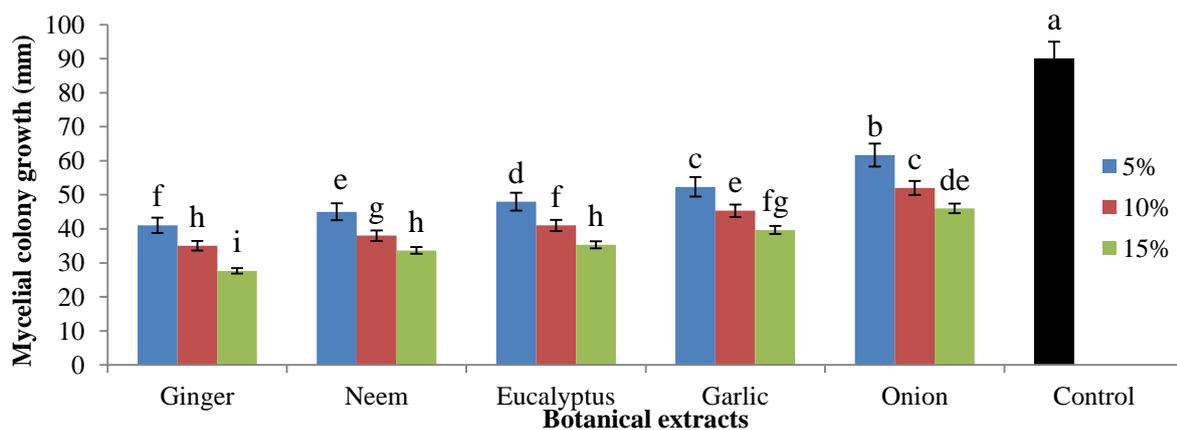


Figure 6: Effect of different botanical extracts on the linear colony growth of *Penicillium expansum* under *in-vitro* conditions.

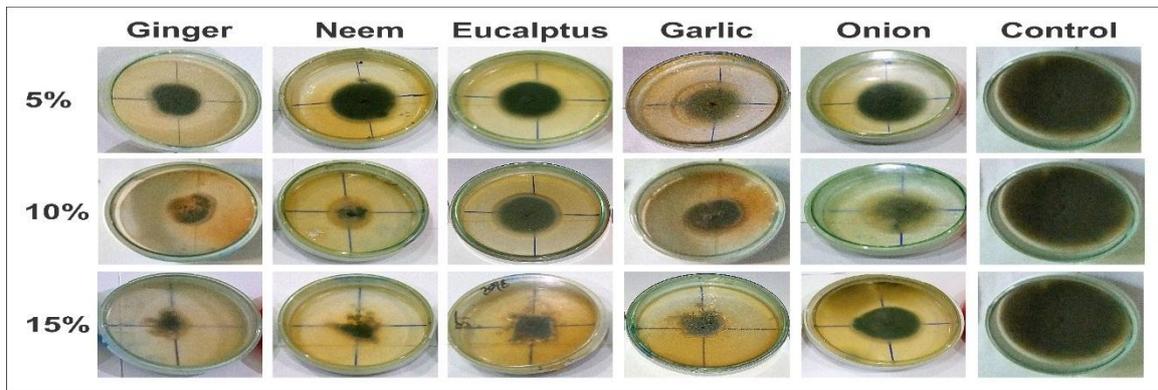


Figure 7: Linear colony growth of *Penicillium expansum* as influenced by botanical extracts under *in-vitro* conditions.

**In-vitro effect of different bio-control agents against *Penicillium expansum*:** The results presenting in Fig-8 and 9 indicates that the minimum linear colony growth of *Penicillium expansum* was observed for *Guignardia* sp. 29.66 mm followed by *Chaetomium* sp. 35.33mm, *Hypo-*

*xylon* sp. 35.66mm, *Fusarium* sp. 40.66mm, *Cryptococcus* sp. 47.66mm when compared with the control, which was 90 mm. Statistically there was significant  $p < 0.05$  difference in the linear colony growth among the bio-control agents and control.

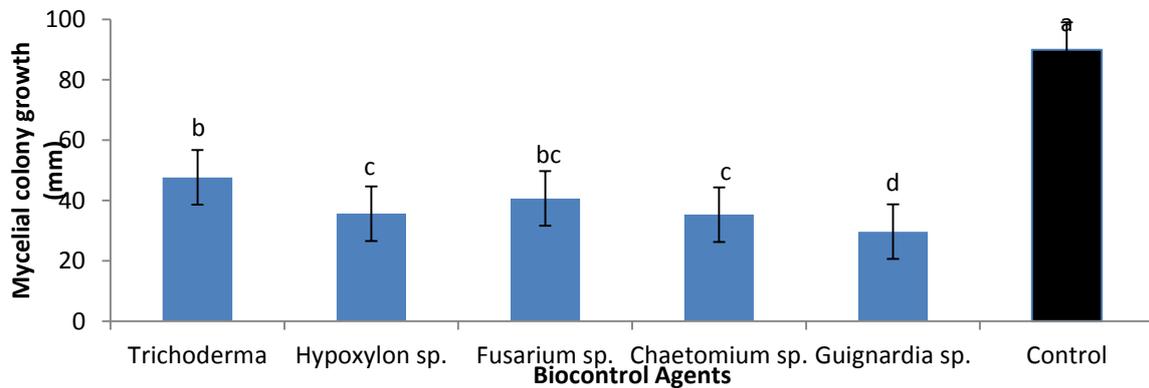


Figure 8: Effect of different biocontrol agents on the linear colony growth of *Penicillium expansum*.

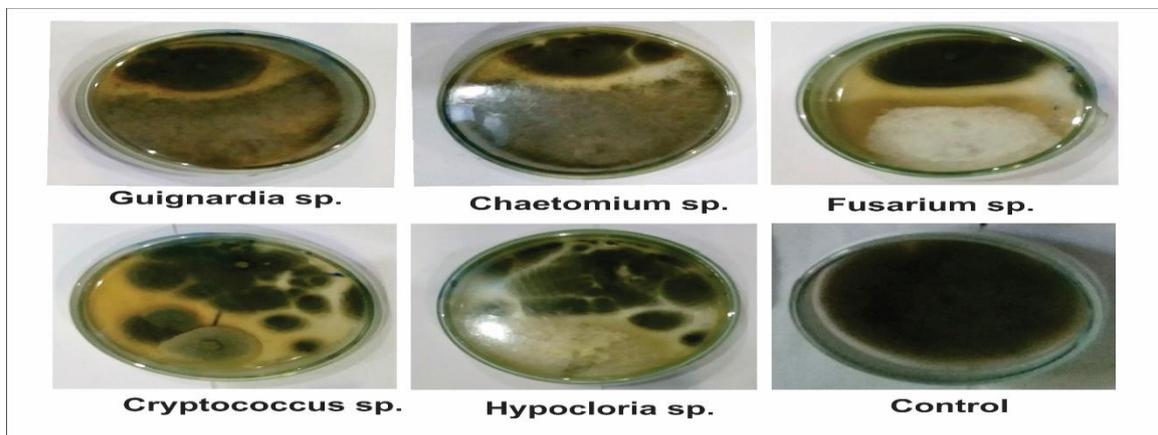


Figure- 9: Linear colony growth of *Penicillium expansum* as influenced by biocontrol agents under *in-vitro* conditions.

**DISCUSSIONS**

It was concluded that chemical fungicide Topsin-M was highly effective to control the linear colony growth of *Penicillium expansum* at 300 ppm. The findings of the current study are in agreement with the study of Revathi and Hemalatha, (2014). They stated that chemical fungicides redu-

ced the fungus growth significantly ( $p < 0.05$ ) better than botanical extracts; however, botanical extracts also showed better response as compared to than control. The effectiveness of botanical extracts in post-harvest storage of marigold can be used at a commercial scale. Latorre (2014) reported that systemic fungicides Hexaconazole showed higher eff-

icacy than Carbendazim, Propiconazole, Difencconazole, Thiophanate methyl and non-systemic fungicides i.e. Mancozeb ranks first for control of fungus than Curzet, Chlorothalonil, Propineb and Copper oxychloride. A similar kind of report was reported by (Carson *et al.*, 2015). Reported that marigold (*Tagetes erecta* L.) cv. 'Crackerjack' is an important commercial ornamental pot and garden flower.

Among the botanical extracts, ginger showed better efficacy against the linear colony growth of *Penicillium expansum* at 15% concentration (Swapnil *et al.*, 2013). They stated neem oil and its leaf extracts significantly ( $p < 0.05$ ) reduced the severity of fruit rots (Terol *et al.*, 2014). Reported that plant extracts as biopesticides act as vital components for the management of this disease. Neem leaf extract gave 58.6% inhibition in radial growth and 56.5% in spore germination at 10% concentration followed by *Ocimum sanctum* which was found effective and gave 54.7% inhibition in radial growth and 50.4% in spore germination over control (Wilson *et al.*, 2011). Revealed that all plant extracts exhibited significantly different mycelial growth inhibition of pathogen. Among them, *J. curcas* leaf extract showed maximum growth inhibition 62.9% followed by *D. strumarium* 55.6%, *A. indica* 51.9%, *M. oleifera* 46.9%, *C. gigantea* 23.45% and *M. alba* 13.6% respectively (Wilson *et al.*, 2011). Revealed that all the concentrations of plant extracts exhibited significantly different in spore germination inhibition of *R. stolonifer* and *A. alternata*. Maximum spore germination inhibition was observed on higher concentration compared to the lower concentration of marigold, garlic and mint, correspondingly (Kusch *et al.*, 2017). Revealed that all the extracts significantly inhibited the mycelial growth at this concentration wherever *Madhuca longifolia* and *Tagetes patula* showed least mycelial growth. However, the leaf extract of Eucalyptus was found to be most effective as compared to other including control for controlling the disease caused by *A. alternata*. An intense study on these leaf extract may help to use them as an effective biopesticides in commercial scale (Terol *et al.*, 2014). Stated that maximum antifungal potential was observed with the extracts of *C. sativa*, which recorded excellent inhibitory activity against *C. lunata* 100%, *A. zinnia* 59.68% followed by leaf extract of *P. hysterophorus* 50% against *A. solani*. Many fungal diseases have been found to attack cucumber. Leaf spot caused by *Alternaria alternata* is an important disease reported to be very destructive diseases which affect the growth, yield and quality of cucumber. The use of chemicals for managing the disease is expensive and often leads

to environmental pollution, development of fungicide resistant strains of the pathogens and upset of the biological equilibrium in soil (Singh *et al.*, 2014).

Among the biocontrol agents, *Guignardia* sp. showed better efficacy against the linear colony growth of *Penicillium expansum*. A similar kind of result was reported by Scordino *et al.*, (2008) who evaluated *Penicillium* species for the control of black rot disease. According to their findings, all the *Penicillium* species remarkably control the colony growth inhibition of fungi compared to control. The disease management generally is done by the chemical control such as fungicides used commonly under field conditions in standing crop, fruit and vegetable plants throughout the year depending upon the intensity of pathogen. Due to over/misuse of fungicides causing harmful effects in human health, disturbing the equilibrium of the ecosystem and reducing the shelf life of fruits and vegetables, the losses reach up to 20% in harvested products in the countries (Cappellini and Ceponis, 1984). Whereas, in developing countries, the losses are rated by about 50% due to poor cultural practices (Eckert and Ogawa, 1985). To overcome the problems due to overuse of fungicides, appropriate cultural and biological control should be practiced during the fruit and vegetable cultivation and also avoid the use of fungicides in field conditions.

### Conclusion

On the basis of present findings, it was concluded that minimum linear colony growth of *Penicillium expansum* was observed at 300 ppm for Topsin-M followed by Melody Duo, Antracol, Alliete, and Cabriotop. The minimum linear colony growth of *Penicillium expansum* was observed at 15% for Ginger followed by Neem, Eucalyptus, Garlic and Onion. The minimum linear colony growth of *Penicillium expansum* was observed for *Guignardia* sp. followed by *Chaetomium* sp., *Hypoxylon* sp., *Fusarium* sp. and *Trichoderma* sp.

### Suggestions

It is suggested that Topsin-M as a fungicide, Ginger extract as botanical and *Guignardia* sp., as biocontrol agent may be used for the control of *Penicillium expansum*, the causal agent of blue mold of apple.

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