Impact Of *Aspergillus* Fungi On Germination Of Chilli Seeds And Its Control

Tharsika Ratnarajah, Gunasingham Mikunthan

Abstract: Chilli fruits are commonly contaminated by Eurotimycetes fungus, *Aspergillus* sp. (Eurotiales: Trichomaceae). This fungus produced white to lime green mycelium; yellowish to green conidia, which size is around 34±0.8µ. This study was carried out to investigate the effect of *Aspergillus* sp. on chilli seed germination. The seeds of the chilli variety MI green were obtained from DATC, Jaffna for using this experiment. Chilli seeds were treated with *Aspergillus* sp., *Trichoderma viride*, *Aspergillus* sp. + *Trichoderma viride*, *Aspergillus* sp. + Neem extract and distilled water as control. Highest germination percentage were observed in T_5 (suspension of *T.viride*) as 73% followed by 65% germination from T_1 (distilled water), 44% from T_3 (suspension of *Aspergillus* sp. and *T.viride*), then T_4 (suspension of *Aspergillus* sp. and neem leaf extract) and T_2 (suspension of *Aspergillus* sp.) showed the germination as 30% and 20%, respectively. The growth inhibition percentage of *Aspergillus* sp. in presence of *T. viride* and *T. harzianum* were calculated by dual culture over control. The percent inhibition of radial growth of *Aspergillus* sp. was 55.35% by *T. viride* and 50.25% by *T .harzianum* in seven days after inoculation. Results show that *T.viride* was superior to *T.harzianum* in restricting the growth of *Aspergillus* sp. on dual culture in PDA.

Keywords: Red chilli, *Aspergillus* sp, *Trichoderma* sp, chilli seeds.

1 Introduction

Chilli (*Capsicum frutescens* L.) is one of the economically important cash crops cultivated in Sri Lanka. Both dry and fresh pods of chilli are considered as most important commercial spices with high nutritional values and use as spice in food and beverages as whole form or powdered form. Moulds are widely distributed as environmental contaminants. Chillies contaminated before processing anywhere in the food chain. Chillies are produced in countries with tropical climates that have high range of temperature, humidity and rain fall. Usually the red chillies are spread out on the ground to dry in the open air where the climatic conditions are ideal for growth of moulds and production of mycotoxins. *Aspergillus* sp. is commonly found on chilli fruits stored in humid region. *Aspergillus flavus* is a predominant component of the mycoflora of red chilli [5]. The occurrence of moulds and mycotoxins can be alleviated by the application of a variety of preventive measures both before and after harvest including appropriate pest and disease control measures and good harvesting drying and storage practices.

2 Materials and methods

2.1 Effect of *Aspergillus* sp. on germination of chilli seeds

The seeds of the chilli variety MI green were obtained from Department of Agriculture, Jaffna for using this experiment. Seeds were selected without any deformities by visual examination. Chilli seeds were treated with *Aspergillus* sp., *Trichoderma viride, Aspergillus* sp. + *Trichoderma viride*, *Aspergillus* sp. + Neem extract and distilled water (Table 1).

- *Tharsika Ratnarajah, Following masters in Post Graduate Institute of Agriculture, University of Peradeniya, Sri Lanka.*
- *Prof. Gunasingham Mikunthan, Professor in Agricultural Biology, Faculty of Agriculture of University of Jaffna, Sri Lanka.*

2.3 Preparation of suspension of *Aspergillus* sp., *Trichoderma* sp and neem leaf extract

Five petri plates of *Aspergillus* sp. , three petri plates (90cm diameter) of *Trichoderma viride* were obtained from seven days old culture. They were dissolved in 450ml and 300ml distilled water, respectively and shaken manually. Then the content was filtered through sterile muslin cloth. Neem leaf extract was prepared from ground the 20g neem leaves in 50ml distilled water. The supernatant was filtered through sterile muslin cloth and used for the experiment.

2.4 Seed treatment of chilli

Twenty-five chilli seeds were taken and dipped into prepared different treatment suspension for ten minutes. Then the treated seeds were placed in petri plates contain whatman filter paper and cotton wool as bed.

Table 1. Combination of seed treatment of chilli

Treatments	Combinations
1	50ml suspension of *Aspergillus* sp.
2	50ml suspension of *Trichoderma viride*.
3	50ml suspension of *Aspergillus* sp. and 50ml suspension of *Trichoderma viride*.
4	50ml suspension of *Aspergillus* sp. and 50ml Neem leaf extract.
5	50ml distilled water.

Three replicates were done in each treatment. Watering was done daily and allowed the seeds for germination. Germination percentage was estimated according to following equation (Atallah, 1983).

$$G\% = \frac{(TNS)-(NNGS)}{} \times 100 \quad \textbf{(1)}$$

TNS
Whereas;
TNS: Total number of seeds
NNGS: Number of non-germinated seeds

2.5 Bio control of *Aspergillus* sp. under *in vitro* condition

Aspergillus sp.p, *T. viride* and *T.harzianum* were inoculated and cultured separately in to PDA medium. Seven days

after the incubation, 4mm agar slugs of *Aspergillus* sp. and *T. viridae* were placed opposite to each other. Three replicates for each set were maintained. Controls were set in single and dual culture of *Aspergillus* sp. and same method was used for *T. harzianum*. The growth inhibition was calculated by using the formula.

$$\frac{(C-T)}{C} \times 100 \qquad \textbf{(2)}$$

Whereas:
C - Growth in control
T - Growth in treatment.
Assessments were made when fungi achieved an equilibrium after which there was no further alteration in the growth. The radial growths of tested pathogens were measured after seven days of incubation period [8], [9], [6].

3 Results and discussion

3.1 Effect of *Aspergillus* sp. on chilli seeds germination

Germination percentage of chilli seeds were examined in different treatment suspension.

Table 2: Mean germination percentage of chilli seed.

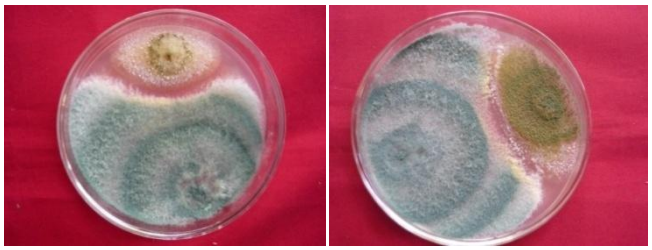
Treatments	Mean germination percentage
T_1- 50ml distilled water	65.33[a]
T_2- 50ml suspension of *Aspergillus* sp.	20.00[d]
T_3- 50ml suspension of *Aspergillus* sp. and 50ml suspension of *Trichoderma viride*	44.00[b]
T_4-50ml suspension of *Aspergillus* sp. and 50ml Neem leaf extract	30.66[c]
T_5-50ml suspension of *Trichoderma viride*	73.33[a]

Values having same letter are not significantly different according to the Duncan mean separation at 95% confidence interval.

Highest germination percentage of chilli seeds were observed in T_5 as 73% followed by 65% germination from T_1, 44% from T_3, then T_4 and T_2 showed the germination as 30% and 20%, respectively. Treatment comparison of T_5, T_1, and T_3 with T_2 had significance difference in their mean germination percentage, exhibited antagonistic effect with T_2 (*Aspergillus* sp.). T*richoderma* sp enhance the seed germination of chilli [2]. *Trichoderma viride* could restrict growth of post-harvest pathogens namely *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus, Fusarium* sp, and *Pencillium* sp [7]. *Azadiracta indica* is known to has antifungal, antibacterial, antiviral, insecticidal, and insect repellent properties [1], [4]. Aqueous extracts of leaves and seeds of *A. indica* inhibit aflatoxin production by *Aspergillus flavus* [10].

3.2 Bio control of *Aspergillus* sp. by *Trichoderma* sp under *in vitro* condition

The growth inhibition percentage of *Aspergillus* sp. in presence of *T. viride* and *T. harzianum* were calculated by dual culture over control. The percent inhibition of radial growth of *Aspergillus* sp. was 55.35% by *T. viride* and 50.25% by *T harzianum* in seven day after inoculation. Result showed that *T. viride* was superior to *T. harzianum* in restricting the growth of *Aspergillus* sp. on dual culture with PDA. The inhibitions of radial growth of *A. niger* (55%), *A. flavus* (51%), and *A. fumigatus* (52%) on par with *T. viride* were present results [7].



A **B**

Figure.1. Colony interaction between the *Aspergillus* sp. with *Trichoderma viride* (A) and *Trichoderma harzianum*(B).

4. Conclusion

Germination percentage of chilli seeds were increased by *Trichoderma viride* (73%) followed by distilled water (65%). 44%,30%,20% germination of chilli seeds were observed in *Aspergillus* sp. + *Trichoderma viride*, *Aspergillus* sp. + Neem and *Aspergillus* sp. treatment suspension, respectively. *Aspergillus* sp. infected seeds had less germination percentage. *T. viride* (55.35%) was superior to *T. harzianum* (50.25%) in restricting the growth of *Aspergillus* sp. on dual culture PDA medium under *in-vitro* condition. Contamination of *Aspergillus* sp. on chilli seeds will drastically reduce the seed germination. Before sowing, seed will treat with *Trichoderma* sp is better method for get more germination percentage.

5. Acknowledgement:

This research is supported by the research project grant NTRP/2012/FS/PG-05/P-02

6. References

[1] Allameh, A., Razzaghi-Abyaneh, M., Shams, M., Rezaee, M. B. and Jaimand, K. 2001. Effects of neem leaf extract on production of aflatoxins and activities of fatty acid synthestase, isocitrate dehydrogenase and glutathione S-transferase in *Aspergillus parasiticus*. Mycopathologia, **154**:79-84.

[2] Asaduzzaman, M., Alam, M. J. and Islam, M. M. 2010. Effect of *Trichoderma* on seed germination and seedling parameters of chilli. Journal of the J. Sci. Foundation, **8** (1&2):141-150.

[3] Atallah, M. M.1983. International mycotoxin Conference 1 Cairo, 19th March (Abst).

[4] Bhatnagar, D. and McCormic, S. P. 1988. The inhibitory effect of neem (*Azadiracta indica*) leaf extracts on aflatoxin synthesis in *Aspergillus parasiticus*. Journal of the American Oil Chemists Society, **65**:1166-1168.

[5] Flannigan ,B., and Hui,S.C. 1976. The occurrence of aflatoxin producing strains of *Aspergillus flavusin* the mould floras of ground spices. Journal of Applied Bacteriology. **41**:411- 418. In Seenappa, M., Stobbs, L.W. and Kempton, A.G. 1980. *Aspergillus* colonization of Indian Red pepper during storage. Phytopathology, **70**:218-222.

[6] Molla, A.H., Fakhru'l-Razi, A., Abd-Aziz, S., Hanafi, M.M. and Allam,M.Z. 2001. *In vitro* compatibility evaluation of fungal mixed culture for bioconversion of domestic waste water sludge. World J. Microbial .Biotechnol., **17:**849-856.

[7] Rajendiran, R., Jegadeeshkumar, D., Sureshkumar, B. T. and Nisha, T. 2010. *In vitro* assessment of antagonistic activity of *Trichoderma viride* against post-harvest pathogens. Journal of Agricultural Technology, **6**(1): 31-35.

[8] Skidmore, A. M. and Dickinson, C. H. 1976.Colony interactions and hyphal interference between *Septoria nodorum* and phylloplane fungi. Trans. Br. Mycol. soc .**66**:57-64.

[9] Stahl, P. D. and Christensen, M. 1992. *In-vitro* mycelia interactions between *Ceratocystis ulmi* and Elm bark saprobes *in vitro* and in vivo. Trans. Br. Mycol. Soc, **86**:93-101.

[10] Zeringue, H. J. Jr. and Bhatnagar, D. 1990. Inhibition of aflatoxin production in *Aspergillus flavus* infected cotton bolls after treatment with neem (*Azadiracta indica*) leaf extracts. Journal of the American Oil chemist Society, **67**:215-216