Study on Trichoderma sp and its metabolites to inhibit the growth and aflatoxin production of Aspergillus flavus

From:Yang-Tze High School

Abstract

There are many cases of waste caused by excessive *aflatoxin* content in peanut products. In this study, by discussing the use of *Trichoderma* to inhibit the effect of aflatoxin, a "new method to effectively prevent the production of *aflatoxin*" was found. First, a qualitative test was performed to confirm whether the *Trichoderma* could inhibit the growth of *aflatoxin* . The second step is to use confrontation culture to screen out the *Trichoderma* that can inhibit the *aflatoxin*. The third step, the *Trichoderma* is cultivated under the environment with *aflatoxin*, and the strain with better tolerance to aflatoxin is selected. The fourth step is to carry out the peanut pods test and use the aflatoxin Total Aflatoxin Rapid Test Kit to detect. The fifth step is to explore the possible factors that the *Trichoderma* strains ETS1-1-2, ETS3-1-8 and ETS4-3-7 inhibit *aflatoxin*. Finally, the strain ETS1-1-2 with the best performance was selected, and its metabolite solution was prepared and tested on fruit pods, etc., to explore the inhibitory effect of *Trichoderma* metabolite solution on *aflatoxin*.

Background

1. **More than 90% of the world's peanuts are grown in Asia, Africa and India. China and Indonesia are the main countries that produce and process peanuts. In recent years, there have been many cases of poisoned caused by eating peanut. And government found that more and more aflatoxin exceed the standard.**
2. **Most of the methods for dealing with aflatoxin in the market have not been widely used due to high cost, difficulty in removal under high temperature, and damage to the nutritional value of crops.**
3. **At present, many studies have confirmed that Trichoderma can be used as a biological control agent to manage a variety of plant diseases, and can effectively control peanut pathogens. In production, Trichoderma can be fermented into spore preparations for soil treatment, which can effectively prevent and control disease.**

Purpose

1. **Exploring whether Trichoderma has inhibitiry effect on Aspergillus flavus**
2. **Select Trichoderma strains with better inhibitory effect on Aspergillus flavus and aflatoxin**
3. **Test the effect of Trichoderma practical application on peanut pots**
4. **Detect the content of aflatoxin inhibition by Trichoderma whether lower than the government limit**
5. **Discussion on the inhitory factors of Trichoderma to Aspergillus flavus**

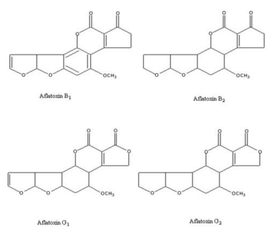
Literature Review

Table 1：General removal method of aflatoxin

Removal method	mechanism of action
Add alkaline substances	Put aflatoxin in an alkaline environment, it will be destroyed by itself
UV irradiation	Utilize ultraviolet light to degrade into less toxic metabolites (Fan et al., 2012)
Add oxidizing chemicals	Hydrogen oxide, ozone and chlorine can be used as oxidants to destroy aflatoxin
heat treatment	After half an hour of above 260°roasting,the aflatoxin will decrease. (Nantou Hospital, 2020)

Table 2：Trichoderma strains inhibit pathogenic germ of peanut

Author	Trichoderma species	Disease name
Meng-Lu Cai et al, 2017	T. virens	**Southern blight** (Sclerotium rolfsii Sacc.)
Chia-Fu Tai, 2014	T. Harzianum T. virens	**Southern blight** (Sclerotium rolfsii Sacc.)
Jing-Hui Wang, 2011	T. harzianum	**Southern blight** (Sclerotium rolfsii Sacc.)
Federico G. Rojo et al, 2006	T. harzianum	**crown rot** (Aspergillus crown rot)



Research Methods

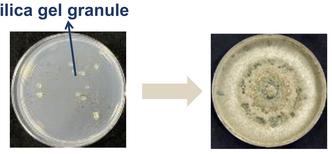
[A1]：Cultured Trichoderma

Step:

1.Pour the silica gel granule in the preservation tube into the WA medium

2.Use a hole punch (0.5mm) to make holes in the growing mycelium, take out the mycelium block, and move it to PDA medium for cultivation

silica gel granule



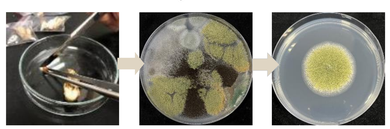
[A2]：Purified Aspergillus flavus

Step:

1.Get **pathogenic germ of peanut** (source: National Formosa University)

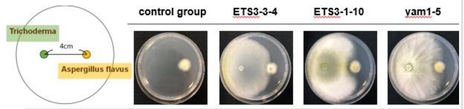
2.Cut off the diseased part of the peanut , Culture in WA medium for 1-2 days, then move to PDA for 3-5 days

3.The isolated Aspergillus flavus were purified and cultured to observe the growth



[A3]Confrontation test

Randomly select *Trichoderma* to confirm *Trichoderma* can inhibition *Aspergillus Flavus*



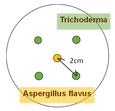
Determine whether the experimental group has an inhibitory effect

All have inhibitory effect

[B1]Inhibition Rate

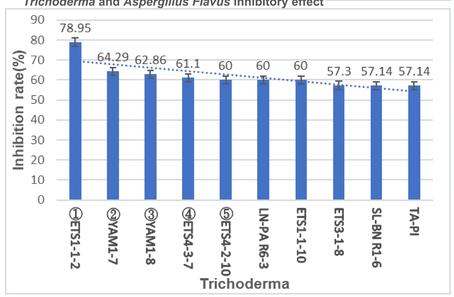
Formula caclucation their inhibition rate

$$\frac{\text{Mycelium of control group average growth length} - \text{Mycelium of text group average growth length}}{\text{Mycelium of text group average growth length}} \times 100$$



(ETS1-1-2)66.55%
(ETS4-3-7)61.10%
(ETS3-1-8) 60.25%

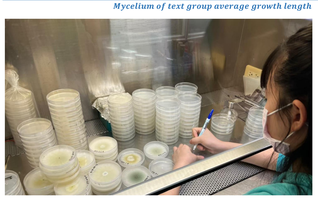
Trichoderma and *Aspergillus Flavus* inhibitory effect



[B2]Passive inhibition rate

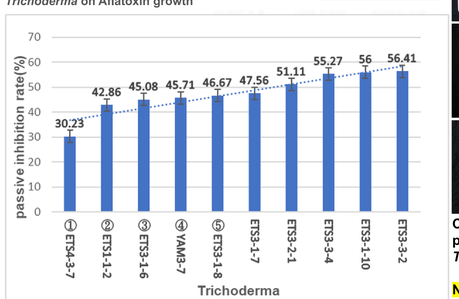
Formula caclucation their inhibition rate

$$\frac{\text{Mycelium of control group average growth length} - \text{Mycelium of text group average growth length}}{\text{Mycelium of text group average growth length}} \times 100$$

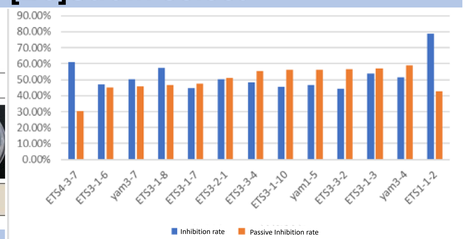


ETS4-3-7 30.23%
ETS3-1-6 45.08%
yam3-7 45.71%
ETS3-1-8 46.67%

Trichoderma on Aflatoxin growth



[B3]Strain select



ETS1-1-2、ETS4-3-7、ETS3-1-8 perform the best

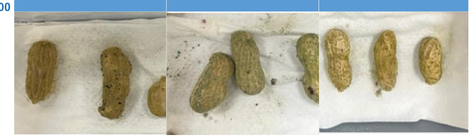
	ETS1-1-2	ETS4-3-7	ETS3-1-8
B1 Inhibition rate	78.95%	61.10%	57.30%
B2 Passive inhibition rate	42.86%	30.23%	46.67%

[C1]Bacteria liquid product

Scrape off the spores	Draw the spores	Put the spores on blood count version	Use the microscope to count the spores

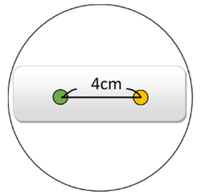
[C2&3]Practical application

Control group	Prevention group	Reprevention group

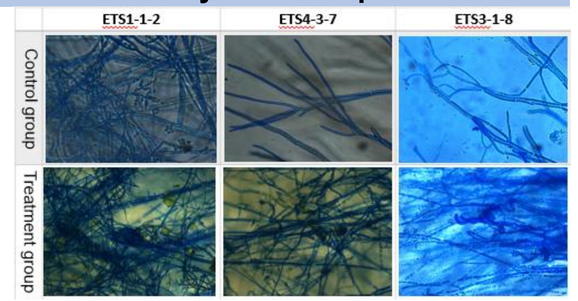


Common peanut pots put in *Trichoderma* liquid	Common peanut pots put in *Trichoderma* liquid, 2days later, put in *Flavus* liquid	Common peanut pots put in *Flavus* liquid, 2days later, put in the *Trichoderma* liquid
Normal conditions	**Prevention before infect**	**Infection before prevent**

[D1] Analyze the inhibitors by microscope

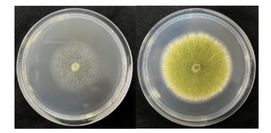


***Trichoderma* mycelium twine *Aspergillus flavus* mycelium**



[D2] Analyze Trichoderma metabolites

Make the medium full of *Trichoderma* metabolites connect on *Aspergillus flavus* and compared with the PDA medium connect on *Aspergillus flavus*



Discussion

A. **The qualitative test results showed that *Trichoderma* has the potential to inhibit the growth of *aflatoxin*, but there is a significant difference in the inhibition effect of each individual.**
B. **The strains with the best inhibition rate of aflatoxin and *aflatoxin* were selected. From the experiments of *Trichoderma* confronting *aflatoxin*, it can be found that *Trichoderma* inhibits *aflatoxin* generally well.**
C. **The preventive test completely inhibited the growth of *aflatoxin* in ETS1-1-2, ETS4-3-7 and ETS3-1-8. The levels of *aflatoxin* were below the limit (15 ppb) in the peanut pods tested in the prevention trial using a Total Aflatoxin Rapid Test Kit for *aflatoxin*. ETS1-1-2 was the most effective in prevention test and treatment test.**
D. **Under the microscope, we can observe the junction of *Trichoderma* inhibiting *aflatoxin*, and we can obviously observe the mycelium of *Trichoderma* entangling *aflatoxin*, and the main inhibiting factors are *Trichoderma* metabolites and mycelium**

Specific contribution

1. **The actual content of aflatoxin in peanuts meets the food standard and the result of the Total Aflatoxin Rapid Test Kid is negative, which can reduce food waste and human harm caused by excessive aflatoxin.**
2. **Compared with the existing aflatoxin treatment methods in the market, the "*Trichoderma* treatment method" is simple and convenient, and lasting effect.**
3. **Excellent effect in preventing peanuts from being infected by *Aspergillus flavus*, and it solves the problem of aflatoxin production during storage.**

Recommendations for Future Work

It is known that the metabolites of Trichoderma strain ETS1-1-2 have an inhibitory effect on inhibiting the growth of Aspergillus flavus. In experiments, extracting its inhibitors has good effects in preventing and treating diseased peanut pods. Commercialization of this Trichoderma strain and diluting its concentrated chemical inhibitors, it can avoid the residue of aflatoxin in food, and will not produce spores and hyphae due to the strain, which will affect the appearance and consumer perception. The scope of application is also wider, not only for peanut pods, but also for processing soybean oil, nut products, milk powder and other foods that easily exceed the standard of aflatoxin.

References

- Nantou Hospital (2008) http://www.nant.mohw.gov.tw/?aid=509&pid=1&page_name=detail&iid=337
- Fan Yangguang, Zuo Kehua, Wei Hengwei, Jin Yuezu, Cai Qingen (2012)。Utilize ultraviolet light to degrade into less toxic metabolites
- Meng-Lu Cai et al, (2017) Study on the biocontrol of peanut southern blight disease by Trichoderma virens
- Chia-Fu Tai, (2014) Study on Correlation between The Capacity of Oxalic Acid Degradation of Trichoderma spp. and Their Growth Inhibition of Sclerotium rolfsii
- Jing-Hui Wang, (2011) Biocontrol Mechanism study in Trichoderma harziamum against Sclerotium rolfsii
- Federico G. Rojo et al, (2006)Biological control by Trichoderma species of Fusarium solani causing peanut brown root rot under field conditions