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Trichoderma Album and Envirolyte-Egypt: New Innovated Methods for the Control of Toxigenic Aspergillus Flavus in the Field Of Corn Plant and Stored-Grains

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Abstract*— The aim of this work was to apply recent ways for the control of toxigenic Aspergillus flavus and aflatoxin contamination in the field and stored-grains of corn. The phytopathogenic fungi (T. album) and Envirolyte (electrochemically activated water) were applied to contaminated soil and stored-corn grains with toxigenic strain of A.flavus as trials to test their capability of destroying the toxigenic A.flavus. For this purpose, 1) Maize plots were used for planting and growing in the contaminated soil with toxigenic A. flavus without any treatment and other plots were treated with spore-culture of T. album, 2) Envirolyte-Egypt diluted solutions (1/100 and 1/250).The results indicated that T. album ($1.5x10^5$ spores.ml^{-1} sterile saline solutions) and Envirolyte-Egypt (at concentration of 1/100) were completely destroyed the toxigenic A. flavus of maize plant in field and in contaminated stored-corn grains.

***Index Terms*—Biocontrol, Phytopathogenic Fungi, Toxigenic Aspergillus, Trichoderma album, Corn.**

I. INTRODUCTION

A mycotoxin (from Greek μύκης (mykes, mukos) "fungus" and Latin (toxicum) "poison") is a toxic secondary metabolite produced by organisms of the fungi kingdom, commonly known as molds [1], [2]. The term 'mycotoxin' is usually reserved for the toxic chemical products produced by fungi that readily colonize crops [1]. Aflatoxins are a type of mycotoxin produced by *Aspergillus* species of fungi, such as *A. flavus* and *A. parasiticus* [3]. The umbrella term aflatoxin refers to four different types of mycotoxins produced, which are B_1, B_2, G_1, and G_2 [4]. Aflatoxin B_1, the most toxic, is a potent carcinogen and has been directly correlated to adverse health effects, such as liver cancer, in many animal species [5],[3]. Aflatoxins are largely associated with commodities produced in the tropics and subtropics, such as cotton, peanuts, spices, pistachios and maize [6],[7],[8],[9],[3],[4]. Contamination by aflatoxin occurs both during maize development and after harvesting [10]. Mycotoxin poisoning in humans and animals occurs through ingestion, inhalation and absorption through the skin [11],[12]. High-level exposure may cause instant death while long-term chronic effects include cancer, mutagenicity and nervous disorders [13]. At the farm level, the real problem is that contaminated maize may appear just like the normal grain without any outward physical signs of fungal infection. Destruction of aflatoxins by conventional food processing is difficult because they are typically resistant to heat and detection is complicated due to limitations in analytical capacity. In this paper, we test two methods for the control of aflatoxin contamination in corn; one of recent development in biological control and the other is innovated one. The phytopathogenic fungi (*T. album*) and *Envirolyte* (electrochemically activated water) were applied to contaminated soil and maize grains (*Zea mays subsp. mays* L) with toxigenic strain of *A.flavus* as a trial to test their capability of destroying the toxigenic strains of *A.flavus*.

II. MATERIALS AND METHODS

Toxigenic A. flavus

Soil samples were collected from a commercial field of maize infected with toxigenic *A. flavus*. Fifteen samples were collected, each of the samples consisted of a mixture of 10 soil samples (5–10 g each) taken from the top 3 cm of soil at different places within the field. The samples were taken in diagonal section at 100 m intervals. Sub-samples of each sample were combined in a paper bag and air-dried for1–2 days at 25–30°C. Samples weighing 100 g were thoroughly mixed passed through a testing sieve (2 mm mesh size) and the soil separated from the debris. Soil samples were stored at 5°C. Enumeration of fungal propagates was carried out on solid medium, using the surface spread method, by blending 10 g soil of each sample with 90 ml 0.1% peptone water solution. Serial dilutions of 10^{-1} to 10^{-3} from each sample and 0.1 ml aliquots were inoculated in triplicate on *A. flavus* agar (AFPA) medium. The Petri dishes were incubated at 30°C for 48 h [14].

Fungal identification

Macroscopic examination of fungal colonies that looked like Aspergillus flavus was subcultured on malt extract agar medium (MEA) for further identification. Aspergillus species were identified according to taxonomic schemes proposed by [13].

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Determination of toxigenic potential of Aspergillus flavus

Aspergillus flavus isolated from soil was tested for their toxicity by plating experiments. Toxigenic potential of pure cultures of Aspergillus flavus following inoculation on yeast extract sucrose medium (20g Sucrose, yeast extract20g made up to 1L with distilled water) (YES) were determined by their fluorescence character in UV light at 365 nm wavelength [15] . These plates were examined at 12, 24, 36, 48, 72hrs, and 4th, 5th and 7th day post inoculation. Both observed and reverse sides of the plates were examined under UV (Black Ray model C50; Ultra- violet product, San Gabriel, Calif).

Entomopathogenic fungi

Trichoderma album. Source: Biozeid (Local) Company-Egypt. It contains 25 x 106 spores per mg. Selective media (Sabaroud agar) rich with antibiotics and growth substances are used for the cultivation of Trichoderma [14],[15],[16] .Haemocytometer was used to count numbers of propagules [17].

Effect of T.album

10 cultivated soil plots (500 g each) were used for planting seeds of maize. Five plots were used for planting and growing maize in the contaminated soil (A. flavus 1-1.5 x104 spore.g-1 soil) without any treatment (G1) and the other five plots (G2) were treated with 100 ml of spore-culture of T. album (1.5x105 spores.ml-1 sterile saline solutions).

Corn grains treatment

Individual solutions of 0 (untreated control, sterile water only), 105 spores .ml-1 of the strain of fungus /100 g grains were prepared in sterile distilled water. Each of the individual lots was sprayed using a Badger 100 artists' airbrush to mist 0.4mL of solution from a particular concentration directly onto the material. For each concentration, the treated material were put in 0.95-L glass jars and hand-tumbled for 30 s to ensure uniform treatment with fungus. The experiments were replicated five times. For each replicate, five individual lots of 100 g were weighed out from grains.

Effect of Envirolyte-Egypt

Envirolyte-Egypt (non toxic, contains various mixed oxidants predominantly hypochlorous acid and sodium hypochlorite [(HClO, ClO2, HClO3, HClO4, H2O2, O2, ClO-, ClO2-, ClO3-, O-, HO2-, OH- - working substances), pH 2.5-3.5, ORP>1150mV, Cactive ~500mg/l.

Maize crop

15 contaminated soil plots (500 g each) were used for planting seeds of maize, divided as the following:

1) Five plots were used for planting and growing maize in the contaminated soil (A. flavus 1x104 spore.g-1 soil) without any treatment (G1),2) Five plots were used for planting and growing maize in the contaminated soil (A. flavus 1x104 spore.g-1 soil) and treated with 100 ml of fogging-sprayed using a Badger 100 artists' airbrush to mist solution from a particular concentration 1\100 (G2), 3)The other five plots were treated with 100 ml of fogging-sprayed of concentration 1\250 (G_3) .

Corn grains treatment

Individual solutions of 0 (untreated control, sterile water only), 105 spores .ml-1 of the strain of fungus /100 g grains were prepared in sterile distilled water. Each of the individual lots was sprayed using a Badger 100 artists' airbrush to mist 0.4mL of spores-solution and then were followed by Envirolyte-Egypt solution from a particular concentration (1/100; 1/250). For each concentration, the treated material were put in 0.95-L glass jars and hand-tumbled for 30 s to ensure uniform treatment with fungus and the disinfectants. The experiments were replicated five times.

Statistical analysis

The obtained data were subjected to analysis according to [18]. Differences between means were done at the 5% probability level, using Duncan's new multiple range test. All statistical analyses were performed using SPSS 12 for Windows.

III. RESULTS

Effect of T.album on A.flavus

Maize crop

Mycological analysis from soil samples showed that the mean colony count of *A.favus* during the pre-planting period was $1x10^4$ spore.g^{-1} soil. While in post-harvest period was $1x10^5$ spore.g^{-1} soil in G_1.

Table 1: Effect of *T.album* on *A.flavus* population in soil during pre-planting and pos-harvesting periods,

Sampling period	Soil sample (mean cfu g^{-1} ±S.E.)		No. of +ve plots		%	
	G_1	G_2	G_1	G_2	G_1	G_2
Pre-planting	$1x10^{4a}$ ±200	$1x10^{4a}$ ±250	5^a	5^a	100^a	100^a
Post-harvesting	$1.5x10^{5a}$ ±300	0^b	5^a	1^a	100^a	20^{b*}

The same letter in the same raw is not significantly different at P<0.05. *: In *post-harvest period was ranged from 0 to $1x10^2$spore / soil (4 plots were negative and one plot positive).* G1: plots were used for planting and growing maize in the contaminated soil (*A. flavus* $1x10^4$ spore.g^{-1} soil) without any treatment;G2: plots were treated with 100 ml of spore-culture of *T. album* ($1.5x10^5$ spores.ml^{-1} sterile saline solutions).

Stored-Grains treatment

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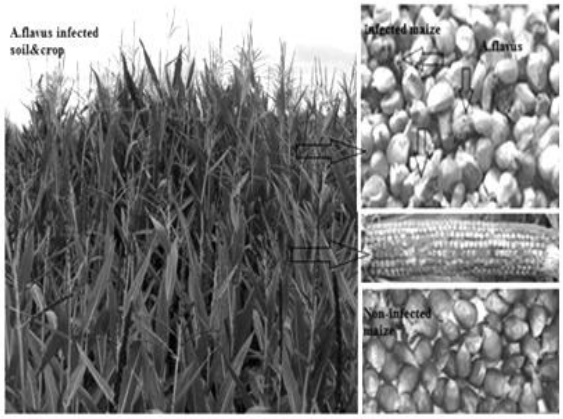

Fig 1: Left fig.: Plots were used for planting and growing maize in the contaminated soil (toxigenic A. flavus 1x104 spore.g-1 soils) without any treatment by T.album. [Other plots (not in figure) were treated with 100 ml of spore-culture of T. album (1.5x105 spores.ml-1 sterile saline solutions)]. Right up & middle figures: Was harvested contaminated soil. Right below figure: was harvested corn grains derived of T.album-treated plots. Mycological analysis from grain samples showed that the mean colony count A.favus before treatment were 1x105 ; 1x104 ; 0; 0 spore.g-1 grains in Ga; Gb; Gc ; Gd ,respectively. While in post-treatment were 1.5x105; 0; 1.5x105; 0 spore.g-1 grains in Ga; Gb; Gc ; Gd ,respectively, as shown in Table 2.

Table 2: Effect of T.album on A.flavus population on corn-stored grains.

Sampling period	cfu g^{-1}			
	Contaminated grains	Non-contaminated grains		
	G_a=A	G_b= A+T.	G_c = A	G_d = A+T
Before treatment	$1x10^{5a}\pm300$	$1x10^{4a}\pm^{250}$	0	0
After treatment	$1.5x10^{5a}\pm 400$	0^{b}	$1.5x10^{5a}\pm^{300}$	0^{b}

The same letter in the same raw is not significantly different at P<0.05. Ga=A: ration contaminated with toxigenic A.flavus; Gb= A+T : ration contaminated with toxigenic A.flavus and treated with T.album; Gc = A: non-contaminated and treated with toxigenic A.flavus; Gd = A+T: : non-contaminated and treated with toxigenic A.flavus and T.album.

Effect of Envirolyte-Egypt

Maize crop

Mycological analysis from soil samples showed that the mean colony count of A.flavus during the pre-planting period was 1.5x104, 1.3x104 and 1.5x104 spore.g-1 soils in G1, G2 and G3, respectively. While in post-harvest period were; 1.5x105, 0 and 1x102 spore.g-1 soil in G1, G2 and G3, respectively.

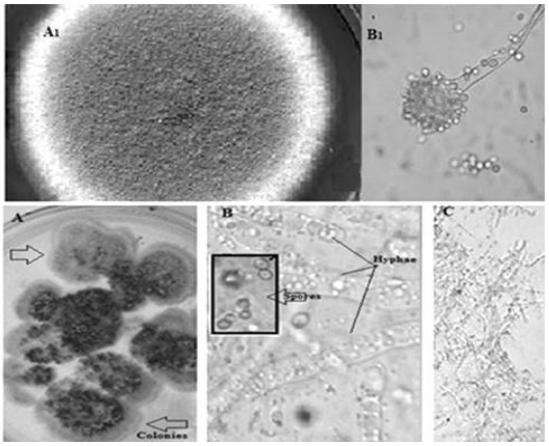

Figure 2: A1: Colonies of toxigenic A.flavus; B: Hyphae of toxigenic A.flavus (X 400). A: Colonies of T.album; B: Hyphae of T. album (X 400); C: Hyphae of T. album (X 100). A: Colonies of T.album; B: Hyphae of T. album (X 400); C: Hyphae of T. album (X 100). planting period was 1x104 spore.g-1 soil. While in post-harvest period was 2 x105, 0 and 1 x102 spore.g-1 soil respectively as shown in Table 3. Envirolyte-Egypt at concentration of 1/100 completely destroyed the toxigenic A. flavus of maize plant in the field.

Grains treatment

Mycological analysis from grain samples showed that the mean colony count before treatment were 1x105 ; 1x104 ; 0; 0 spore.g-1 grains in Ga; Gb and Gc ,respectively. While after treatment were 1.5x105a, 0b and 1.5x105a spore.g-1 grains in Ga; Gb and Gc, respectively, as shown in Table 4. Envirolyte-Egypt at concentration of 1/100 completely destroyed the toxigenic A. flavus of maize plant in the field.

Table 3: Effect of Envirolyte-Egypt on Aspergillus Flavus population in soil during preplanting and harvesting periods.

Sampling period	Soil sample cfu g^{-1}			No. of +ve plots			%		
	G_1	G_2	G_3	G_1	G_2	G_3	G_1	G_2	G_3
Pre-planting	$1.5x10^{4a}\pm150$	$1.3x10^{4a}\pm200$	$1.5x10^{4a}\pm150$	5^{a}	5^{a}	4^{a}	100^{a}	100^{a}	80^{a}
Post-harvesting	$2x10^{5a}\pm300$	0^{b}	$1x10^{2a}\pm100$	5^{a}	1^{a}	2^{a}	100^{a}	20^{b*}	40^{a}

The same letter in the same raw is not significantly different at P<0.05. *: In *post-harvest period was ranged from 0 to 1x10^{2}spore / soil (4 plots were negative and one plot positive).* G1: plots were used for planting and growing maize in the contaminated soil (*A. flavus* 1.5 x10^{4} spore.g^{-1} soil) without any treatment;G2: plots were treated with 1/100 *of Envirolyte-Egypt;* G3: plots were treated with 1/250 *of Envirolyte-Egypt.*

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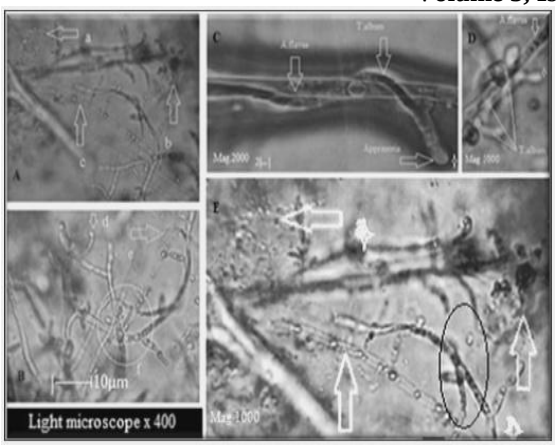

Figure 3: A&B: Photograph: 3:A&B: Light microscopic photograph (X400) showing mycelial and conidial destruction of *A .flavus* by *T.album*. A: *Trichoderma* is attached and coils around the pathogen; *A .flavus* (a, b& c); B: It forms the appresoria (d&e). The following step consists of the production of CWDEs and peptaibols, which facilitate both the entry of *Trichoderma hypha* into the lumen of the parasitized fungus and the assimilation of the cell-wall content (f). **C, D&E:** Scanning Micrograph; C: Showing: *T.album* is attached; it coils around the pathogen *A.flavus* and forms the appresoria (**D**). The following step consists of the production of CWDEs and peptaibols, which facilitate both the entry of *Trichoderma hypha* into the lumen of the parasitized *fungus* and the assimilation of the cell-wall content. **E,** showing mycelia and conidial destruction of *A .flavus* by *T.album* (Arrows).

Table 4: Effect of Envirolyt-Egypt on Aspergillus Flavus population in stored-corn grains.

Sampling period	cfu g^{-1} Contaminated corn-grains			No. of +ve plots			%		
	G_a	G_b	G_c	G_a	G_b	G_c	G_a	G_b	G_c
Pre-treatment	$1x10^{5a}$ ±250	$1x10^{4a}$ ±150	$1.5x10^{4a}$ ±200	5	5	5	100	100	100
Pos-treatment	$1.5x10^{5a}$ ±300	0^b	$1.5x10^{5a}$ ±350	5	0^b	0^b	100	0^b	0^b

The same letter in the same raw is not significantly different at P<0.05. G_a: corn contaminated with toxigenic *A.flavus*; G_b: corn contaminated with toxigenic *A.flavus* and treated with *1/100 Envirolyt- Egypt*; G_c: corn contaminated with toxigenic *A.flavus* and treated with *1/250 Envirolyt-Egypt.*

IV. DISCUSSION

In our study, we apply recent development in biological control of aflatoxin contamination. The phytopathogenic fungi (*T. album*) applied to contaminated soil and contaminated corn-grains with toxigenic strain of *A.flavus.*

T. album was succeeded in the destroying of *A.flavus* contaminated plant and corn grains. Mycological analysis of treated soil samples (post-harvesting) and also treated grains, were showed a highly significant reduction (P<0.05) in fungal count of *A.flavus*, as shown in Table 1and 2. Numerous organisms, including bacteria, yeasts and nontoxigenic fungal strains of *A. flavus* and *A. parasiticus*, have been tested for their ability in controlling aflatoxin contamination. Great successes in reducing aflatoxin contamination have been achieved by application of non toxigenic strains of *A. flavus* and *A. parasiticus* in fields of cotton, peanut, maize and pistachio [19]. *Trichoderma* strains exert biocontrol against fungal phytopathogens either indirectly, by competing for nutrients and space, modifying the environmental conditions, or promoting plant growth and plant defensive mechanisms and antibiosis, or directly, by mechanisms such as mycoparasitism. These indirect and direct mechanisms may act coordinately and their importance in the biocontrol process depends on the

***Trichoderma* strain [23], [23],[24].**

Trichoderma, a fungus, helps humans in many ways; it is used to give denim a stone-washed look. It is used to increase the digestibility of barley that is mixed into chicken feed. But it is also, scientists have found, especially good at eating "bad" fungi and considered a welcome tool in the agriculturalist tool box [20]. In developing countries, fungal diseases that attack and destroy crops are a major problem [3], [4]. Antagonists of phytopathogenic fungi have been used to control plant diseases, and 90% of such applications have been carried out with different species of the fungus

***Trichoderma* [20].**

Mycoparasitism involves morphological changes, such as coiling and formation of aspersorium- like structures, which serve to penetrate the host and contain high concentrations of osmotic solutes such as glycerol [20]. Trichoderma attaches to the pathogen with cell-wall carbohydrates that bind to pathogen lectins. Once Trichoderma is attached, it coils around the pathogen and forms the appresoria (Fig.3 and 4). The following step consists of the production of CWDEs and peptaibols [25], which facilitate both the entry of Trichoderma hypha into the lumen of the parasitized fungus and the assimilation of the cell-wall content. The chitinolytic system of Trichoderma comprises many enzymes and the list of its components is rapidly being updated as new enzymes and genes are reported. Chitinases are divided into 1, 4-β-acetylglucosaminidases (GlcNAcases), endochitinases [20]. Trichoderma harzianum secretes alpha-1,3-glucanases when it is grown on polysaccharides, fungal cell walls, as a carbon source (simulated antagonistic conditions) [26]. Hyphal penetration of solid materials may be accomplished by pressure alone but is usually accompanied by various sorts of chemical attack. As the hyphal tip pushes into its environment it releases substances that soften or even dissolve material in its path. This combination of hyphen tip growth and chemical release is the very essence of the fungal organism; everything else is secondary [20]. Electrolysed water or electro-chemically activated water solution is produced by the electrolysis of ordinary water containing dissolved sodium chloride. Electro-activated water possesses high ability to penetrate through biological barriers. Although

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the present study did not examine the exact mechanism of action, we believe that the biological effect of Envirolyte-Anolyte against fungi were due to the combined action of hydrogen ion concentration, oxidation-reduction potential and dissolved chlorine. Envirolyte-Anolyte is a strong acid, but it is different to hydrochloric acid or sulphuric acid. These acids have a strong degree of ionization, and when oxidation occurs, H+ is used and new H+ is generated [27].

VI. CONCLUSION

The phytopathogenic fungi (*T. album*) and electrochemically activated water (*Envirolyte-Egypt*) were applied to contaminated soil and corn grains (*Zea mays subsp. mays* L) with toxigenic strain of *A.flavus* as a trial to test their capability of destroying the toxigenic strains of *A.flavus*.

T. album ($1.5x10^5$ spores.ml^{-1} sterile saline solutions) and *Envirolyte-Egypt* (at concentration of 1/100) were completely destroyed the toxigenic *A. flavus* of maize plant in field and in contaminated stored-corn grains.

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REFERENCES

[1] N.W. Turner, S.Subrahmanyam, ands.A. Piletsky, "Analytical methods for determination of mycotoxins: a review, "Anal Chim Acta, Vol., 632 (2), pp., 168–80, 2009.

[2] J.L. Richard," some major mycotoxins and their mycotoxicoses—an overview," Int J Food Microbial, Vol., 119 (1–2), pp., 3–10, 2007.

[3] M.L. Martins, H.M. Martins and F.Bernardo," Aflatoxins in spices marketed in Portugal," Food Addit Contam, Vol.,18 (4), pp,.315–9,2001.

[4] P. Bayman and J.L Baker, "Ochratoxins, a global perspective," Mycopathologia,Vol., 162 (3), pp.,215–23,2006.

[5] R.K. Garber and P.J. Cotty," Formation of Sclerotia and Aflatoxins in Developing Cotton Bolls Infected by the S Strain of Aspergillus flavus and Potential for Biocontrol with an Atoxigenic Strain,"Phytopathology, Vol.,87(9),pp.,940-945, 1997.

[6] H.P. Van Egmond, "Mycotoxins: regulations, quality assurance and reference materials," Food Additives and Contaminants, Vol., 12,pp., 321–330, 1995.

[7] P.J. Cotty, "Aflatoxin-producing potential of communities of Aspergillus section Flavi from cotton producing areas in the United States," Mycological Research, Vol., 101,pp.,698–704, 1997.

[8] B.W. Horn and J.W. Dorner,"Soil population of Aspergillus species from section Flavi along a transect through peanuts-growing region of the United States," Mycologia,Vol., 90,pp.,767–776, 1998.

[9] D.T. Wicklow, C.E. McAlpin and C.E. Platis," Characterization of the Aspergillus flavus population within an Illinois maize field," Mycological Research, Vol., 102, pp., 263–268, 1998.

[10] A.Nesci and M. Etcheverry," Aspergillus section Flavi populations from field maize in Argentina," Letters in Applied Microbiology, Vol., 34, pp.,343–348, 2002.

[11] D.L. Park and B. Liang ,"Perspectives on aflatoxin control for human foods and animal feed, "Trends in Food Science and Technology, Vol.,4 (10), pp.,334-348 ,1995.

[12] J.M. Wagacha and J.W. Muthomi,"Mycotoxin problem in Africa: Current Status, implications to food safety and health and possible management strategies," International Journal of Food Microbiology, Vol., 124,pp., 1-12,2008.

[13] J.I. Pitt and A.D. Hocking,"Fungi and Food Spoilage," Vol. II, edited by London, Blackie Academic and Professional, 1997.

[14] M.S. Goettel and D.G. Inglis ,"Fungi: Hyphomycetes. In: Manual of techniques in insect pathology," Lacey LA, edited by Academic Press, London, UK, pp, 213-249, 1997.

[15] K.Yabe, Y. Ando, M. Ito and N. Terakado,"Simple Method for Screening Aflatoxin Producing Molds by UV photography," Applied and Environmental Microbiology, Vol.,53, pp.,230–234, 1987.

[16] V.C. Beilharz, D.G. Parberry and H.J.S. Wart,"Dodine: A selective agent for certain soil fungi," Transactions of the British Mycological Society, Vol.,79, pp.,507-511, 1982.

[17] C.M. Ignoffo,"The fungus Nomuraea rileyi as a microbial insecticide," In: Microbial control of pests and plants diseases, edited by Academic Press, London, pp, 513-38, 1980.

[18] O.W. Snedecor and W.O. Cochran, "Statistical Methods," 7th edn., Iowa University Press, Ames, Iowa, 1982.

[19] Y.N Yin, L.Y. Yan, J.H. Jiang and Z.H. Ma ,"Biological control of aflatoxin contamination of crops,"J Zhejiang Univ Sci, Vol.,B 9 (10), pp.,787–92, 2008.

[20] Tagrawal and A.S.Kotasthane,"Chitinolytic assay of indigenous Trichoderma isolates collected from different geographical locations of Chhattisgarh in central India,"Springerplus,vol., 1(1), pp.,73,2012.

[21] E. Monte,"Understanding Trichoderma: between biotechnology and microbial ecology,"Int Microbial, Vol., 4,pp.,1-4, 2001.,"

[22] T. Benítez, A.M. Rincón, M.Carmen Limón and A.C. Codón,"Biocontrol mechanisms of Trichoderma strains," International microbiology, Vol., 7,pp.,249-260, 2004.

[23] H.B. Singh, B.N. Singh, S.P. Singh and B.K. Sarma,"Exploring different avenues of Trichoderma as a potent bio-fungicidal and plant growth promoting candidate- an overview." Rev Plant Pathol, Vol., 5, pp., 315–426, 2012.

[24] P.K. Mukherjee, A.K. Mukherjee and S. Kranthi,"Reclassification of Trichoderma viride (TNAU), the most widely used commercial biofungicide in India, as Trichoderma asperelloides,"Open Biotechnol J,Vol., 7,pp.,7–9, 2013.

[25] C.R. Howell,"Mechanisms employed by Trichoderma species in the biological control of plant diseases: the history and

ISSN: 2277-3754
ISO 9001:2008 Certified
International Journal of Engineering and Innovative Technology (IJEIT)
Volume 3, Issue 6, December 2013

evolution of current concepts," Plant Dis, Vol.,87,pp.,4-10,2003.

[26] H .Ait-Lahsen, A. Soler, M. Rey, J. de La Cruz, Monte E and A. Llobell,"An antifungal exo-alpha-1,3-glucanase (AGN13.1) from the biocontrol fungus Trichoderma harzianum," Appl Environ Microbiol, Vol.,67(12), pp.,5833-5839, 2001.

[27] A. Iwasawa, Y. Nakamura and T. Mizuno,"Antiviral effect of aqua-ionized water,"Clinical Microbiology (in Japanese),Vol., 20,pp., 231-236, 1993.