

# An overview of Fusaric acid production

T.D.Rani, Savitha Rajan, L.Lavanya, S.Kamalalochani,\*B.Bharathiraja.

## Abstract

Fusaric acid (FA) is a mycotoxin produced by the *Fusarium* species, among which the most high yielding was reported to be *Fusarium oxysporum*. It is moderately toxic to animals. It has antibiotic, insecticidal and pharmacological activity. *Fusarium* species are found worldwide in soil as both pathogenic and non-pathogenic strains. Large concentrations of fusaric acid reduce growth of root and root tubers. The biosynthesis of fusaric acid involves condensation reaction of polyacetate and aspartic acid units. The assay involves HPLC, TLC, Mass spectroscopy and NMR techniques. Fusaric acid acts as an enzyme inhibitor, dopamine agent and nucleic acid synthesis inhibitor. As an orally active agent, it may have potential role in the treatment of head and neck squamous cell cancer (HNSCC).

**Keywords:** Fusaric acid, *Fusarium oxysporum*, dopamine, HNSCC.

## Introduction

Fusaric acid (FA) is a host non-specific toxin. The high production of which has been correlated with the Virulence of plant Pathogenic strains of *Fusarium* spp. FA (5-butylpicolinic acid) was first discovered during (Paterson *et al.*, 1991) laboratory culture of *Fusarium heterosporum* nees by Yabuta *et al.* It has a natural contaminant or mycotoxin accumulating during infection in corn and cereal grains is extremely toxic to animals and human beings by enhancing toxicity of other *Fusarium* metabolites EG-: Trichothecenes (Stepfen *et al* 2005). It is not only moderately toxic to animals but also has antibiotic, insecticidal and pharmacological activity in both brain and pineal neurotransmitter and metabolites are affected (Baron *et al.*, 1996). It cause wilt disease symptoms in pepper, corn and used to select with resistance in plant. (Peterson *et al.*, 1991).

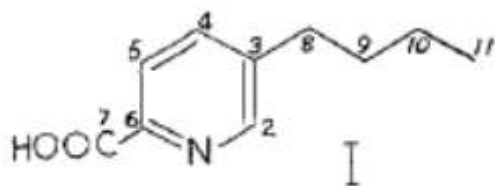


Fig1: Structure of Fusaric acid

The most expansive producer of this toxin is *Fusarium oxysporum* or, its special forms *F.Spp.lycopersici* (Stefan *et al.*, 2005) *Fusarium* spp are ubiquitous fungi found in soil world wide as both pathogenic and non pathogenic strains. FA could elicit various plant defense response at 100nm without toxic effect. Large FA concentration reduces root and root-hair growth and induce a rapid transient membranes Hyperpolarisation (Brahim *et al.*, 2006).It represses the production of PCN (Phenazine 1- Carboxamide) and of the quorum sensing signal N-hexanoyl-L-homoserine lactones (C6-HSL). It is toxic for Eukaryotes and Prokaryotes involved in fungal defense against *Pseudomonas* Spp. Biocontrol strains by repressing the production of antifungal metabolites. (Tjeerd *et al.*, 2005) FA Showed higher nematocidal activity against *B.xylophilers*. (Hyeok *et al.*, 2007). The biosynthesis of FA involves the condensation reaction involving a polyacetate unit and Aspartic acid. (Hill *et al.*, 1966) The specific role of FA are Picolinic acid and involvement of ethylene in disease development is not known (David *et al.*, 1978) Mycelial growth could not be used to measure or estimate the production of FA. FA causes rot of potato tubers (Sonja *et al.*, 1996). FA has a Tumoricidal activity for head and neck squamous cell cancer. (HNSCC). In plant, most of the studies and FA reported toxic effect at concentration greater than 10<sup>-5</sup> M. FA can be involved in Fungal pathogenicity by decreasing cell viability. It could induce typical early defense response such as reactive O<sub>2</sub> species. (ROS prdn). (Brahim *et al.*, 2006). FA is detected using HPLC, TLC, Mass spectroscopy, NMR.

## Properties

The phytotoxic pathogenicity factor FA represses the production of 2,4 diacetyl phloroglucinol (DAPG) a key factor in the antimicrobial activity of biocontrol strain *Pseudomonas fluorescense*. The effect of FA, a dopamine beta hydroxylase inhibitor, was determined on aggression motor activity and brain monoamines at dose of 3.2 to 60 mg/kg. FA is a mycotoxin with low to moderate toxicity, which is, of concern, since it might be synergistic with other occurring mycotoxin. FA decreases contractile response elictor with nor-epinephrine, histamine, serotonin, acetylchdine and KCl. FA does not interfere with Dopamine uptake.

- FA represses the PCN production under different environmental condition.

- Moderate FA doses (50-100µm) induce apoptotic features, while high FA dose (>200µm) stimulates necrosis.
- Decarbonylation of FA gives  $\text{Co}_2(\text{C}-7)$  and 3-butyl pyridine, which is oxidized with  $\text{KMnO}_4$  to nicotinic acid.
- The activity is found mainly in positions 2, 3, 5, 9&11 of fusaric acid. The low activity at C-2 & C-3 of pyridine ring & presence of pyridine ring & presence of activity at C-4 & C-7 are consistent with the participation of 4C Krebs's cycle acid (or) its equivalent (hill *et al.*, 1966)
- It has been shown to produce both ethylene & ethylene like symptoms (David *et al.*, 1978)
- Fusaric acid can be used as selective agent (Wenzel *et al.*, 1990)
- FA show nematocidal activity agent *B.xylophilus* (Hyeok *et al.*, 2007)
- FA inhibits dopamine B-hydroxylase (Hiroyoshi *et al.*, 1969).
- Some other fusarial mycotoxins are trichothecene, deoxynivalenol, *F.graminearum* toxic, T-2 and beauverin. (Hongsheng *et al.*, 2008)

## Applications

FA acts as an enzyme inhibitor, dopamine agent and nucleic acid synthesis inhibitor.

### 1. FA, An antitumor agent

FA can chelate divalent cations, esp., Zn & inactivate Zn finger proteins involved in DNA repair & protein synthesis. FA has tumoricidal activity for head & neck squamous cell cancer (HNSCC). *In vivo* studies demonstrated that daily intralesional therapy for 1 month showed reduced onset of growth & overall growth of cell. Thus FA appears to have a tumorstatic/tumoricidal effect on HNSCC. As an orally active agent, it may have potential role in treatment of HNSCC. (Ruda *et al.*)

### 2. FA, An antiarrhythmic agent

Hydrochloride beta-(N,N-diethylamino) ethylamide of FA (DAEA) exerted an antiarrhythmic activity in adrenaline-induced arrhythmic in rats. DAEA single pretreatment in dose of 1-5 mg/kg prevented the disorder of rhythm & conductivity in most animals. A pronounced Antiarrhythmic effect was manifest at doses of 2 & 4mg/kg of DAEA. It prevents the development of arrhythmic in 50% of animals in a dose of 1.7mg/kg. (Savina *et al.*)

### 3. Effect of FA on tardine dyskinesia & mental state in psychogeriatric patients

The effect of FA 150-450mg daily on tardine dyskinesia & mental state was studied in 15 chronic psychogeriatric patients. FA significantly relieved orofacial dyskinesia, tremor & rigidity & its improved the mental state of patient (BPRS). Akineria & Anxiety were not altered. (Viukari *et al.*)

### 4. Effect of FA ( An hypotensive agent) on blood pressure of rabbit

When 20mg/kg of FA was injected intraperitoneally into rabbits, rats, cats,(or) dogs. Decrease of blood pressure was observed from about 30

min to 6 hrs after injection. Increase in dose decreases the blood pressure in great level with increasing FA concentration there was marked decrease of nor-epinephrine in angiovascular system is thought to be the cause of hypotensive effect. (Hiroyoshi *et al.*, 1969)

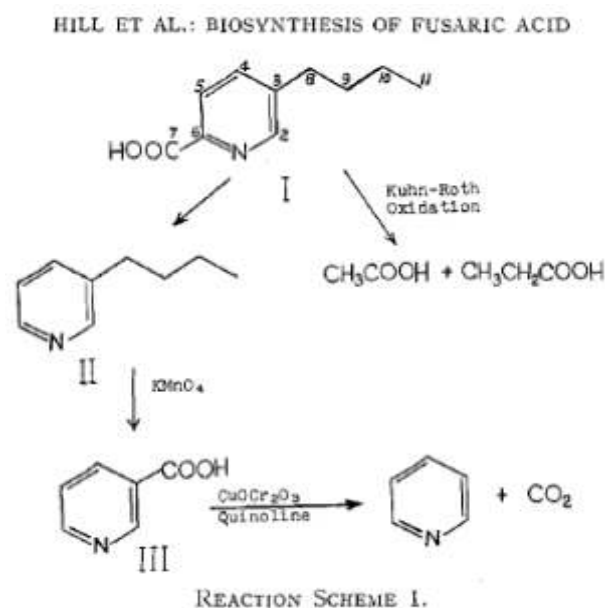
### Other applications

- Administration of alpha-methyl-para-tyrosine (AMPT) & FA helps the withdrawal in human's addicted to narcotics (or) amphetamines.
- FA strongly inhibited root & leaf cell function physiologically responsible for fusarium wilt of watermelon. ( Hongsheng *et al.*, 2008)
- Fusaric acid induce endogenous ethylene production in tomato cutting. (David *et al.*, 1978)
- FA is used to examine the cell death in saffron roots.
- FA increases the mitochondrial & plasma membranes permeability (Leili *et al.*, 2006)
- FA is used for selecting resistance in barley plants (Luz *et al.*, 1990)
- FA is known as an inhibitor of metal-containing oxidative enzymes, mycotoxin and antibiotics (hyeok *et al.*, 2007).
- It is used as a marker compound for *Fusarium* contamination of grains (Bacon *et al.*, 1996)

### Negative Effects

FA causes necrotic spots on leaf blade, Shriveling & drying of leaves and shriveling & wilting of stem & petioles in tomato cutting (Kazuyuki *et al.*, 1996)

Deoxynivalenol (DON) is a fusarium trichothecene mycotoxin, the consumption of which causes growth depression, loss of appetite, vomiting and lesions of intestinal tract (Nannemaches *et al.*, 1991)



Reaction scheme 1: I; Fusaric acid, II; 3-Butylpyridine, III; Nicotinic acid.

FA is toxic to various plants, fungi and bacteria.

FA causes wilt disease symptoms in pepper, corn etc (Luz *et al.*, 1990)

FA cause rot in potato tubers.

### Biosynthesis of Fusaric acid from $^{14}\text{C}$ -labelled acetate in *Gibberella fujikuroi*:

Leete had suggested that the molecule may be formed biologically from a branched chain derivative from five acetate units.

Vining had proposed a slightly different condensation involving a polyacetate unit and aspartic acid.

### Conditions of culture

The fungus was maintained on malt agar (or) difco potato dextrose agar slants at  $4^{\circ}\text{C}$ , with regular subculturing every 2 months. Inoculum was prepared by transferring a suspension of the mycelium to 125 ml Erlenmeyer flasks containing sterile, water saturated rice. After 5 days in the dark, the conidia were harvested by shaking the mixture with sterile, distilled water (60ml). This spore suspension was transferred to a 250 ml Erlenmeyer flask and incubated on a shaker in the dark for 24 h. Ten millilitres of this inoculum was added to 250 ml of Czapek's medium in 1 litre Erlenmeyer flasks, and the fungus was grown in the dark in shake culture. Czapek's medium has the following composition (g/l); Sucrose, 40; Sodium nitrate, 3; Monopotassiumphosphate, 1; Magnesium sulfate, 0.5; Potassium chloride, 0.5; Ferrous sulfate, 0.01; all in distilled water. The pH of the medium was adjusted to 5.5 before autoclaving.

### Administration of labeled compounds

The fungus from shake culture that was 3 days old was collected by low-speed centrifugation. The harvest from two culture flasks was resuspended in Czapek's medium (50ml) and used to inoculate fresh medium (200ml). Six flasks were normally used for each tracer experiment. Aqueous solution of sodium acetate- $1\text{-}^{14}\text{C}$  (or) sodium acetate- $2\text{-}^{14}\text{C}$  were prepared and divided equally between the six flasks. After the flasks were shaken for 24hrs in the dark, they were removed and the fusaric acid was extracted from the culture filtrate.

### Assay of radioactivity

Samples were counted in a Nuclear-Chicago model 725 liquid scintillation counter using as solvents either (a) toluene containing 0.4% 2,5-diphenyl oxazole (PPO) and 0.005% 1,4-bis-2-(5phenyl oxazolyl)-benzene (POPOP) or (b) dioxane-ethyl cellosolve (5:1) containing 5% naphthalene, 0.4% ppo and 0.01% POPOP. Sample activities were determined in duplicate, with a counting error of 5% or less in each determination.

## Materials and methods

### Microorganism

*Fusarium oxysporum*, *Gibberella fujikuroi*, *Fusarium moniliforme*, *Fusarium verticilloides*, *Fusarium arthrosporioides*.

### Incubation period

Cultures were incubated for 7 days at  $24^{\circ}\text{C}$  on a rotary shaker (180 rpm).

### Recovery

Fungal biomass was collected by centrifugation for 15 minutes. One part of supernatant was filtered and stored. In addition, the supernatant was acidified to pH 2 and shaken vigorously for 1 minute. The organic phase was separated from aqueous phase by filtering and brought to dryness in vacuo.

### Assay

HPLC, Mass spectroscopy, Thin layer chromatography, NMR, Gas chromatography are used for assay.

### *Fusarium oxysporum*

#### HPLC

The residue was dissolved in 1ml of methanol and analyzed by HPLC equipped with a reverse phase column packed with nucleosil 120 - 5 C18 and set at  $50^{\circ}\text{C}$ . The samples were eluted with linear methanol. The fraction containing the FA standard from the HPLC was collected, and the presence of FA was qualitatively and quantitatively confirmed by Mass spectroscopy on a TSQ 3000 triple quadrupole mass spectrometer with electrospray ionization. (Regina *et al.*, 2002).

### *Gibberella fujikuroi*

#### GC-MS

The presence of FA in the corn samples was confirmed by GC-MS of the trimethylsilyl ester of an authentic standard on a Hewlett-Packard 5890 series II Gas chromatograph with an HP-5970 mass-selective detector. Additional confirmation of FA was performed by chromatography with an authentic standard of FA developed on pre-coated TLC sheets. (C.W. Bacon *et al.*, 1996).

### *Fusarium moniliforme*

#### HPLC

Preparative HPLC separations of the FA analogs were carried out on a Prep LCI cartridge. Samples were eluted with water-acetonitrile (90:10) at 100ml/min, and the eluent was monitored with a refractive index detector at a sensitivity of 20.

#### TLC

Crystalline FA, DHFA and the diacid analog of FA, DOAFA used as TLC standards were purified by HPLC and recrystallisation until they gave a single spot on TLC plates coated with 0.25 mm thick silica gel 60F-254 with fluorescent indicator.

### Mass Spectrometry

Low resolution mass spectra was obtained by chemical ionization with isobutane as the reagent gas in a Finnigan 4535/TSQ. High resolution electron impact mass spectrometry was determined at 70 eV with a

Nuclide 12-90 DF instrument. Samples were introduced with a direct insertion probe.

### NMR

Proton 1H and carbon13 (<sup>13</sup>C) NMR spectra were recorded at 300 and 75 MHz respectively in deuterium oxide on a Bruker WM -300 instrument with sodium 3-tri methyl silyl propionate-2, 2, 3, 3, d4 as the internal standard.

### *Fusarium verticilloides*

### TLC

Samples were ground and extracted with methylene chloride and analyzed by a modified TLC as described by Bacon et al. After evaporating methylene chloride to dryness, the residue was re dissolved in 3ml ethanol and applied on silufol plates together with FA standard. The plates were developed in n-butanol: acetic acid: ethyl acetate: water (3:2:2:2,v/v). Plates were subsequently dried at 80°C and FA was detected under UV light. FA content was quantified

fumonisin B-1 and fusaric acid measured by injection into fertile chicken egg. *Mycopathologia* **129**:29-35.

Bacon, C. W., J. K. Porter, W. P. Norred, and J. F. Leslie. 1996. Production of fusaric acid by *Fusarium* species. *Appl. Environ. Microbiol.* **62**:4039-4043.

Bangera, M. G., and L. S. Thomashow. 1999. Identification and characterization of a gene cluster for synthesis of the polyketide antibiotic 2,4-diacetylphloroglucinol from *Pseudomonas fluorescens* Q2-87. *J. Bacteriol.* **181**:3155-3163.

Booth, C. 1971. The genus *Fusarium*. Commonwealth Agricultural Bureau, London, England.

Braun, R. 1960. Über Wirkungsweise und Umwandlungen der *Fusarinsäure*. *Phytopathol. Z.* **39**:197-241.

Capasso, R., A. Evidente, A. Cutignano, M. Vurro, M. C. Zonno, and A. Bottalico. 1996. Fusaric and 9, 10-dehydrofusaric acids and their methyl esters from *Fusarium nygamai*. *Phytochemistry* **41**:1035-1039.

Chakrabarti, D. K., and K. C. B. Chaudhary. 1980. Correlation between virulence and fusaric acid production in *Fusarium oxysporum* *Fusarium oxysporum* f. sp. carthami. *Phytopathol. Z.* **99**:43-46.

Davis, D. 1969. Fusaric acid in selective pathogenicity of *Fusarium oxysporum*. *Phytopathology* **59**:1391-1395.

Delany, I., M. M. Sheehan, A. Fenton, S. Bardin, S. Aarons, and F. O'Gara. 2000. Regulation of production of the antifungal metabolite 2,4-diacetylphloroglucinol in *Pseudomonas fluorescens* F113: genetic analysis of pHIFpHIF as a transcriptional repressor. *Microbiology (Reading)* **146**:537-546.

Dowd, P. F. 1988. Toxicological and biological interactions of the fungal

metabolites fusaric acid and kojic acid with xenobiotics in *Heliothis zea* and *Spodoptera frugiperda* (J. E. Smith). *J. Chem. Ecol.* **15**:249-254.

Dowling, D. N., and F. O'Gara. 1994. Metabolites of *Pseudomonas* involved in plant disease. *Trends Biotechnol.* **12**:133-141.

Duffy, B. K., and G. Défago. 1997. Zinc improves biocontrol of *Fusarium* crown and root rot of tomato by *Pseudomonas fluorescens* and represses the production of pathogen metabolites inhibitory to bacterial antibiotic biosynthesis. *Phytopathology* **87**:1250-1257.

Duffy, B. K., and G. Défago. 1999. Environmental factors modulating antibiotic and siderophore biosynthesis by *Pseudomonas fluorescens* biocontrol strains. *Appl. Environ. Microbiol.* **65**:2429-2438.

spectrophotometrically on a specord M 40 spectrophotometer.

## References

Abbas, H. K., S. O. Duke, W. T. Shier, F. A. Badria, C. M. Ocamb, R. P. Woodward, W. Xie, and C. J. Mirocha. 1997. Comparison of ceramide synthase inhibitors with other phytotoxins produced by *Fusarium* species. *J. Nat. Toxins* **6**:163-181.

Abbas, H. K., C. J. Mirocha, T. Kommedahl, R. F. Vesonder, and P. Golinski. 1989. Production of trichothecene and non-trichothecene mycotoxins by *Fusarium* species isolated from maize in Minnesota (USA). *Mycopathologia* **108**:55-58.

Bacon, C. W., J. K. Porter, and W. P. Norred. 1995. Toxic interaction of

Dunne, C., I. Delany, A. Fenton, and F. O'Gara. 1996. Mechanisms involved in biocontrol by microbial inoculants. *Agronomie* **16**:721-729.

Egli, T. A. 1969. Studies on the influence of heavy metals on *Fusarium oxysporum* f. sp. *lycopersici* and progress of tomato wilt. *Phytopathol. Z.* **66**:223-253.

Fenton, A. M., P. M. Stephens, J. Crowley, M. O'Callaghan, and F. O'Gara. 1992. Exploitation of gene(s) involved in 2,4-diacetylphloroglucinol biosynthesis to confer a new biocontrol capability to a *Pseudomonas* strain. *Appl. Environ. Microbiol.* **58**:3873-3878.

Gapillout, I., M. L. Milat, and J. P. Blein. 1996. Effects of fusaric acid on cells from tomato cultivars resistant or susceptible to *Fusarium oxysporum* f. sp. *lycopersici*. *Eur. J. Plant Pathol.* **102**:127-132.

Gäumann, E., S. Naef-Roth, and H. Kobel. 1952. Über Fusarinsäure, ein zweites Welketoxin des *Fusarium lycopersici* Sacc. *Phytopathol. Z.* **20**:1-38.

Heeb, S., Y. Itoh, T. Nishijyo, U. Schnider, C. Keel, J. Wade, U. Walsh, F. O'Gara, and D. Haas. 2000. Small stable shuttle vectors based on the minimal pVS1 replicon for use in gram-negative, plant-associated bacteria. *Mol. Plant-Microbe Interact.* **13**:232-237.

Jullien, M. 1988. Effects of the *Fusarium* spp. toxins and selection of crude toxin resistant strains in mesophyll cell cultures of *Asparagus officinalis*. *Plant Physiol. Biochem.* **26**:713-722.

Keel, C., and G. Défago. 1997. Interactions between beneficial soil

#### About the Authors:

T.D.Rani, Savitha Rajan, L.Lavanya, S.Kamalalochani,  
Bioprocess Laboratory, Department of biotechnology,  
Arunai Engineering College,  
Thiruvannamalai-606603

#### For Correspondence

B.Bharathiraja.  
Senior lecturer, Department of biotechnology,  
Arunai Engineering College,  
Thiruvannamalai-606603.  
E-mail address: btrbio@gmail.com.

*Just Published*

## A Practical Manual for BASIC IMMUNOTECHNIQUES



Title	: A Practical Manual for Basic Immunotechniques
Author(s)	: Dr.M. Ravi Ph.D., Dr. Solomon F.D. Paul Ph.D.
Publisher	: Samanthi Publications Pvt. Ltd.
ISBN	: 978-81-906565-0-4
Size	: 28 cm. X 21.5. Cm.
Binding	: Hard Bound
Price	: Rs.525/-



Order your copies.  
Send DD/Cheque/M.O. in favour of  
Samanthi Publications Pvt. Ltd.  
Payable at Chennai.  
Free delivery against  
full payment (Rs.525/-).

**Samanthi Publications Pvt. Ltd.**  
No. 170, Kakkam Colony,  
Nungambakkam  
Chennai - 600 034.  
Ph : 2817 5693 / 2817 5694  
E mail : info@samanthi.in  
www.samanthi.in

**Topics Covered :** • Qualitative Immunotechniques • Quantitative Immunotechniques  
Production of Polyclonal Antibodies • Production of Monoclonal Antibodies

**Highlights of the Manual :** • Covers most Basic Immunotechniques that can be employed for a variety of applications • Meets the requirements of Graduate students, Postgraduate Students, Researchers & Technicians • Written in simple language with each chapter having a 'Notes' component that focuses on the finer details and critical points of each technique • Backed by excellent colour illustrations and Images that help in easier Understanding and practice of Basic Immunotechniques • Useful Annexure that include one for the preparation of Commonly Required Buffers

**Trade enquires solicited**

**Samanthi  
Lab Manuals for Life Science**