Journal of Ecobiotechnology 2011, 3(8): 01-05
ISSN: 2077-0464
www.scholarjournals.org

www.journal-ecobiotechnology.com

JEBT-Microbial Biotechnology

Host – pathogen Interaction between the Soil Borne Fungi, *Fusarium moniliforme* and Maize Plants (*Zea mays* L.)

N. Swarnakumari[1], D. Ramanan and M. Kanniappan[2*]

[1]*Agricultural Research Station, Tamil Nadu Agricultural University,Virinjipuram, Vellore, Tamil Nadu. India.*
[2]*Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai. Tamil Nadu. India.*

Article Info

Article History

Received : 29-03-2011
Revised : 19-06-2011
Accepted : 20-06-2011

*Corresponding Author

Tel : +919444084530

Email: microkanni@gmail.com

Abstract

Maize is one of the important food crop, it is succumbed to different types of biotic and abiotic constrains. A study was carried out to understand the mode of infection of *F. moniliforme* on maize. The observations revealed that the fungi can infect seeds, leaves, stalks and roots. Seed germination was inhibited by the pathogen infection. Inoculation of pathogen on leaves produced necrotic lesion which resembles leaf blight disease. While, the seedlings expressed toppling symptom, the stem portion above the soil surface showed rotted appearance similar to collar rot on fifth day after inoculation. When the *F. moniliforme* was inoculated into stalks, lesion development (6.5cmx0.5cm) was observed and also the fungi travelled from the inoculated node to the next node. In maize, Bio-chemical changes in healthy and *F. moniliforme* infected leaves were assessed. Protein, total phenol, peroxidase and polyphenoloxidase concentration was higher in infected plants when compared to uninfected samples. The pH of necrotic cells usually lower and in turn peroxidase activity was assumed to be higher. A positive correlation was observed between phenol accumulation and polyphenol oxidase activity (r = 1). This paper describes the methodology and results of the above said experiment.

Key Words: Host, Gibrella miniiformis, Peroxidase, Maize, Pathogenesity, seedlings

Introduction

Maize (*Zea mays*.L) is one of the important remunerative food crops of tropical and subtropical regions of the world [1]. It is grown in wide range of environmental conditions due to its adoptability. This constrains cause considerable yield reduction. Among different biotic factors, insect pests and diseases play a vital role in affecting the productivity. *Fusarium moniliforme* is a problem in maize for the reason that the fungus be able to produce mycotoxins, [2]. Among the variety of pathogens, *Fusarium* is considered as a devastating fungal menace of maize. Fungi of the genus *Fusarium* are abundant in nature and widely distributed [3]. Many of this causes plant diseases. The species *F. moniliforme* attacks member of the family Gramineae, it is pathogenic to sugarcane, sorghum, maize, rice etc. In maize, *F. moniliforme* infection cause seed rot, ear rot, stalk rot and seedling blight. *Fusarium* stalk rot incited by *F. moniliforme* is known to cause substantial yield loses [4]. At the time of harvest, *F. moniliforme* can be found in plants or crop residue but does not survive for long periods in the soil [5]. In addition to its effects on yield and seed quality, the infection can be detrimental to grain quality [6]. A variable pathogenicity of the fungus has been reported with additional pathogen and stress factors [7]. Chlamydospores are survival structures for many soil fungi and are usually produced under certain environmental conditions. The fungus over winters as chlamydospore-like structures and mycelium on plant debris and on seeds [8]. The infection may occur early in the season (possibly at the seedling stage as a result of planting infected seed) and the fungus grows systemically, producing symptoms during the later stages of plant development or the infection occurs later in the growing season. The infection process occurs when the fungus invades tissue directly or through wounds. Common points of entry are roots and stalks at the base of leaf sheaths. Commonly, *F. moniliforme* species is present in seed and becomes active in stalk tissues when the plant approaches maturity or is injured. Spore masses of this fungus are as light-pink powders on the leaf sheaths. There are important a study on pathogenicity [9, 6] of the disease is usually not noticeable until late in the growing season, but the fungus may be present in the corn plant long before this. Researchers have been unable to determine exactly how or when the fungus enters the plant. Evidence suggests it comes in through the roots, leaf sheaths or axillary buds. As the plant nears maturity the fungus decays the interior tissues in the lower portions of the stalk and causes the pith to become soft and spongy, resulting in deterioration of the vascular system. The actual grain weight may be reduced by 5-20 per cent in standing corn harvest [10]. With this background a detailed study was under taken to understand the host-pathogen relationship between *F. monilifome* and maize plants.

Materials and methods

Pathogen *F. moniliforme* was isolated from wilt infected tissues of sugarcane (Fig. 1a) and identified as *F. moniliforme* (Gene bank accession HQ009872).

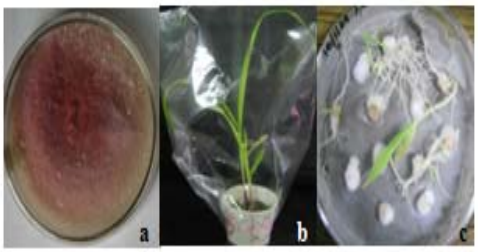

Fig.1 a. Pure culture of Fusarium moniliforme, b. Fusarium moniliforme spores sprayed on maize seedlings, c. Fusarium moniliforme spores on maize seeds

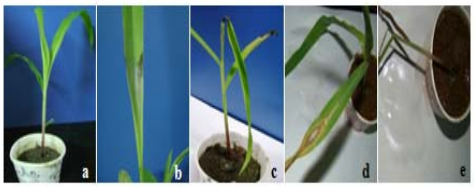

Fig. 2 Influence of *F. moniliforme* causing disease symptoms on maize seedling, a. Control, b. Mycellial colonization on leaf, c. Toppling of seedling, d. Leaf lesion, e, Damping off.

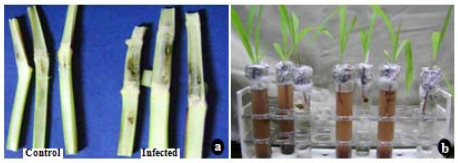

Fig. 3a. Influence of *Fusarium monilforme* causing disease symptoms on maize stalks, b. *F. moniliforme* on the roots maize plant

Pathogenicity Tests: The disease causing ability of *F. moniliforme* was assessed in maize plant by different method of inoculation. The methods are furnished below.

Pathogenicity on seeds: Petriplate and paper cup methods were tried to understand the influence of *F. moniliforme* seeds and seed rot disease of maize. Two sets of sterile petriplates (150 mm dia) were taken. Two layers of tissue papers were placed in the plates and wet with sterile water. Seeds soaked with *F. moniliforme* were placed on the wet tissue paper and incubated for seven days. A control was maintained using seeds soaked in sterile water as check. The observation on mycelial colonization of seeds was recorded (Fig. 1c). The paper cups (100 ml) were taken filled with sterile soil. The surface sterilized seeds were soaked in spore suspension (15×10^6 spores/ml) over night. The seeds soaked in *F. moniliforme* spore suspension (15×10^6 spores/ml) were sown into the cups. It was then incubated for ten days. A control was maintained using seeds soaked in sterile water instead of spore suspension. Watering was given as and when required.

Pathogenicity on seedlings: Paper cups (100 ml) were taken and filled with sterile soil. Maize seeds were sown into the cups. The seedlings were allowed to grow for two weeks. Seedlings were sprayed with *F. moniliforme* spore suspension (15×10^6 spores/ml) at the rate of 2 ml per seedling. Then the seedlings were covered with polythene cover to maintain humidity (Fig. 1b). The whole setup was incubated under room temperature (27±1º C). The observations on leaf lesion and seedling mortality were recorded at 24h intervals. A control was maintained by spraying 2 ml of sterile water. Five replications were maintained for each treatment.

Pathogenicity on stalks: Maize plant was cultivated in the pots under glass house condition. Forty days aged healthy plants were selected. A small puncher was made on the stalks. *F. moniliforme* spore suspension (15×10^6 spores/ml) was inoculated into the punctures using surgical syringe and it was incubated for 15 days. A control was maintained with sterile water inoculation. The observation on lesion development was recorded. The maize stalks were split open to measure lesion development. The infected tissue (30mm x 5mm) from inoculated stalks were collected and surface sterilized using 0.2% (w/v) sodium hypochlorite and 0.1% (w/v) streptomycin sulphate. After sterilization the tissue were placed on the PDA media under aseptic condition. These plates were incubated for eight days under room temperature (27±1º C) to re-isolate the disease causing fungus.

Pathogenicity on roots: Hydrophonic method was followed to assess the influence of *F. moniliforme* on root infestation and root disease of maize. Maize seedlings were grown is small cups for root development. Clean test tubes (150 ml) were taken and filled with 45 ml of *F. moniliforme* spore suspension

(15 x 10^6 spores/ml). Three weeks old maize seedlings were pulled out from cups cleaned using tap water. Root tips of seedlings were cut and placed into the test tube containing *F. moniliforme* spore suspension. A cotton plug was made to enhance firm stand of the seedlings. The top of the test tubes were covered with aluminium foil. It was then incubated for five days under room temperature (Fig. 3b). A control was maintained with sterile water as a check. Four replications were maintained for each treatment. Roots were cut to 1 cm in length and stained with cotton blue stain to observe the fungal penetration.

Biochemical analysis

Samples of infected and uninfected tissues of maize leaves were collected to estimate protein, enzymes, and total phenol. One gram of sample was weighed and utilized for each biochemical parameters.

Estimation of protein: One gram of sample was homogenized with 2 ml of 0.1 M Sodium phosphate buffer (pH 7.0). It was centrifuged at 10000 rpm for 20 min. The 100 µl of supernatant was taken and made up to 1ml using G.D. water. To the sample 5 ml of CCB-G250 was added and OD was read at 595 nm. A blank was prepared without the addition of samples. The OD was compared with BSA standard to quantify protein [11].

Estimation of total phenol: One gram of samples was homogenized with 10 ml of 80% (v/v) ethanol. The samples were centrifuged at 20000 rpm for 20 min. The supernatant was collected, the residue was extracted five times the volume of 80% (v/v) ethanol and then centrifuged. The supernatant was collected to dryness. The residue was made up to 50 ml using G.D. water from that 0.5 ml, 1.0 ml of samples were taken separately and made up to 3 ml using the G.D. water. To this mixture 0.5 ml of 1 N Folin ciacalteau phenol reagent was added and allowed for 3 min. After the incubation period 2ml of Sodium carbonate 20% (w/v) was added. The solutions were mixed thoroughly and kept in boiling water bath for exactly 1 min. It was cooled at room temperature (27±1 º C) and an absorbance was recorded at 650nm. A blank was prepared by adding all the mentioned ingredients without samples. The OD values were compared with pyrocetachol standard [12]. One gram of the above mentioned samples was homogenized with 2 ml of 0.1 M sodium phosphate buffer (pH 7.0) at 4º C. The samples were ground and centrifuged for 20 min at 10000 rpm under 4ºC. The supernatant was used as crude enzyme extract for assay of peroxidase and polyphenol oxidase.

Assay of peroxidase (PO): Assay of PO activity was carried out as per the procedure [13], the reaction mixture 2.5 ml consisted of 0.25% (v/v) guaiacol in 0.1 M sodium phosphate buffer (pH 7.0) and 0.1 ml Hydrogen peroxide. 0.1 ml of enzyme extract was added to initiate the reaction. The absorbance at 470 nm was recorded for every 30 sec for 3 min. A blank was prepared by adding all the ingredients without samples. PO activity expressed the increase in absorbance at 470 nm/min/g of fresh sample. Polyphenol oxidase activity was determined in the reaction mixture consisted of 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5) and 200 µl of enzyme extract. To initiate the reaction 0.3 ml of 0.01 M catechol was added and absorbance was read at 495 nm [14]. The activity of PPO was expressed as changes in absorbance in units/min/g of fresh sample.

Results and Discussion

Pathogenicity Study

Seed germination was inhibited by the pathogen infection. The number of seeds germinated was 36% lower in comparison with the healthy ones. The seed germination was not affected by the presence of *F. moniliforme* var. *subglutinans*, but cold and humid soil the fungi F. *moniliforme* var. *suglutinans*, and *Rhizoctonia solani*, caused reduction in corn seed germination [15]. The seedlings of maize were inoculated with *F. moniliforme* spore suspension and incubated for disease development. The observation was recorded at 24h intervals for five days. Lesion development was observed on leaves and stem (Table 1). Lesion on the leaves: The observation revealed that the leaf surface was colonized at 48h interval (Fig. 2b). Necrotic lesion was observed on third day after inoculation (Fig.1). The lesion was initially yellow in colour then it turned to brown with gray center (Fig. 2d). The lesion had yellow margin at advance stage (120h). Disease development was similar to seedling blight. The stalk of the seedlings was picked up infection at 72h interval. A lesion was developed on the stalk and resulted in breakage of the stem. This finally led to toppling symptom of seedling (Fig. 2c). The stem portion above the soil surface expressed a rotted appearance similar to collar rot on fifth day after inoculation (120h).

Damping off appeared on fifth day after inoculation (Fig. 3e). Due to the collar rot, the seedling was damped off touching the floor. Similarly on maize (Zea mays) the fungus causes seedling blight as well as root, stalk, ear and kernel rot [16]. The pathogenicity study results of the present investigations are in accordance with the conclusion [16]. They reported that the *F. moniliforme* fungal pathogen may cause seedling blight, stalk, and ear rot. They also found that the dry weights of infected seedlings were markedly reduced compare to un-inoculated control seedlings.

Table 1. Systemic infection at different time intervals due to *F. moniliforme* inoculation in maize seedling

Symptom	24h	48h	72h	96h	120h
Mycelial development	-	+	+	+	+
Leaf lesion	-	-	+	+	+
Toppling	-	-	-	-	+
Collar rot	-	-	+	+	+
Damping off	-	-	-	-	+

(+) Symptom observed (-) No symptom

Table 2. Lesion development due to *F. moniliforme* inoculation on stalks

Parameters	Lesion development
Lesion length (cm)	6.50
Lesion width (cm)	0.55
Number of nodes	1.75

Pathogenicity on stalks: The stalks of maize plants were inoculated with *F. moniliforme* spore suspension. The lesion development was recorded on 15 days after inoculation. Necrotic lesions were observed when the inoculated internodes were split opened (Fig. 3a). Different parameters viz., lesion length, lesion width and number nodes crossed were recorded (Table 2). The lesion was 6.5 cm in length and 0.55 cm in width and the pathogen traveled from the inoculated node to the next node. The mean number of nodes infected by the pathogen was more than one. The numbers of nodes infected were counted. No lesion development was observed in the stalks inoculated with sterile water. Only physical damage due to puncture holes was seen. They observed lesion development on stalk of different varieties [4]. The disease symptom described by them is similar to the current observation. The infected maize stalks were used to isolate the *F. moniliforme*. The artificially produced disease should yield the same pathogen on resolution as per Koch postulates theory. Hence the re-isolation of *F. moniliforme* from the infected tissues was carried out. The plates inoculated with the infected tissues showed that lilac colour mycelial growth. The microscopic observation confirmed the species. The mycelia growth was observed inside the epidermal cells of roots. The conidial spores also been observed on the root surface. The hyphal structures were penetrated inside the cells. These observations confirmed that the pathogen can cause root rot. *F. moniliforme* fungus colonized all the underground parts of a plant but was found primarily in lateral roots and mesocotyl tissues [17]. They also confirmed the root rotting.

Table. 3 Biochemical Changes in infected and uninfected tissue samples

Tissue sample	Protein (mg/g sample)	Total phenol concentration (mg/g sample)	peroxidase activity (units/g sample)	Polyphenol oxidase activity (units/g sample
Uninfected	2.70±0.06	1.74±0.04	0.059±0.001	1.942±0.04
Infected	6.19±0.12	3.79±0.08	0.623±0.01	4.032±0.08

Biochemical Changes

The biochemical parameters viz., protein concentration, accumulation of phenol, peroxidase and polyphenol oxidase activities was estimated. Biochemical parameters viz., protein, total phenol, peroxidase and polyphenol oxidase showed that there was a difference between infected and uninfected tissues. The results showed that there was a significant variation in protein concentration among pathogen infected and uninfected plants. It was s observed to be more in infected leaves (6.19/mg/g). The protein concentration in uninfected leaf was about (2.70 mg/g). The protein concentration was increased in infected plant compared to uninfected plant

(Table. 3). Accumulation of phenol in pathogen infected and uninfected plants were estimated by using FCR as phenol equivalent. The phenol concentration of infected leaves was 3.79 mg/g and in uninfected leaves was 1.74 mg/g. The total phenol content is high in infected leaves due to necrotic cells (Table 3). Peroxidase activity was observed once in 30 sec interval for three min. The specific activity/3 in was calculated by applying substrate extinction co-efficient. The result indicated that there was significant variation in PO activity in the infected and uninfected tissues. The results were given in Table 2. The higher peroxidase activity was recorded in infected leaves (0.623 units/g) and lower activity was observed in uninfected leaves (0.059 units/g). The pH of plant cells influences the PO activity. It is more active at low pH. The pH of necrotic cell usually lower and in turn peroxidase activity was assumed to be higher in these cells. The results of the present study agreed with this assumption. The important role of polyphenol oxidase is to oxidize polyphenol in the phenoloic complex. Most phenol occurs in plant tissues in bound form, which contain mono and poly phenols. Accumulation of mono phenols is an important criterion for resistance. The activity of PPO was estimated in infected and uninfected tissues of leaves. The results are furnished in Table 4. Higher PPO activity was observed in infected leaves compared to uninfected leaves. This is may be due to the higher accumulation of total phenols. The enzyme activities were higher in infected plants which show that the pathogen penetration activates the elicitor reaction. This facilitates the pathogen to develop in the cells and cause lesion formation. The results of current study also revealed that the total phenol accumulation is more in infected tissues which in turn resulted in the formation of brownish lesion on leaf tissues. The correlation co-efficient (r) was worked out to understand the relationship exist between PPO activity and phenol accumulation. The statistical analysis showed that there was a positive correlation (r=1) occurred between PPO activity and phenol accumulation. This investigation has concluded the efficient of *F. moniliforme*, which is showed many symptoms of diseases, so crop rotation of the field is not applicable for this pathogen affected field.

Acknowledgement

My heartful thanks to E. I. D parry (India) Ltd, HR Manager, Assistant General Manager and R & D department staff's. My greatful thanks to General Manager of R & D division for providing me the lab facility to carryout my thesis work.

References

[1] Willington Bressan and Jose Edson Fontes Figueiredo. 2002. Efficacy and dose-response relationship in bio-control of *Fusarium* disease in maize by *Streptomyces* spp. Euro. J. Plant Pathol. 120:311-316.

[2] Bacon, C. W. and P. E. Nelson. 1994. Fumonisin production in corn by toxigenic strains of *Fusarium moniliforme* and *Fusarium proliferatum*. J. Food Protection. 57:514-521.

[3] Moss, M. O. and J. E. Smith. 1984. The Applied Mycology of *Fusarium*. Cambridge University Press.

[4] Bohra., R. S. Rathor and Jain, M. L. 2001. Management of *Fusarium* stalk rot of maize caused by *F.moniliforme* Sheldon. J. Mycol. Plant pathol. 31(2):245-247.

[5] Headrick, J. M. and J. K. Pataky. 1988. Resistance to kernel infection by *Fusarium moniliforme* in inbred lines of sweet corn and the effect of infection on emergence. Plant Disease. 73:887-892.

[6] Munkvold, G. P., D. C. McGee and Carlton, W. M. 1997. Importance of different pathways for maize kernel infection by *Fusarium moniliforme*. Phytopathology. 87:209-217.

[7] Velluti, A., S. Marin., L. Bettucci., A. J. Ramos and Sanchis, V. 2000. The effect of fungal competition on colonization of maize grain by *Fusarium moniliforme, F. proliferatum* and *F. graminearum* and fumonisin B-1 and zearaleone formation. Inter. J. Food Microbiol. 59:59-66.

[8] Nyvall, R.F. and Kommedahl. T. 1970. Saprophytism and survival of *Fusarium moniliforme* in corn stalks. Phytopathology 60: 1233-1235.

[9] Andrews, E.A. and R.L. Janes. 1950. Stalk rot of corn in Michigan 1949. Plant Dis. Reptr. 34: 207-208.

[10] Hooker, A. L. and M. P. Britton. 1962. The effect of stalk rot on corn yield in Illinois. Plant Disease Reporter. 46:9-13.

[11] Bradford's, M. M. 1976. A rapid and sensitive method for the qualification of micrograms, Qualitative of protein utilizing the principle of protein dye-binding method. Anal. Biochem. 72:248-254.

[12] Sadasivam, S. and A. Manickam. 1996. Phenol estimation. Plant Biochemistry. 67-69 pp.

[13] Hammerschmidt, R. and J. Kuc. 1982. Lignification as a mechanism for induced systemic resistance in cucumber. Physiol. Molecul, Plant Pathol. 20:61-71.

[14] Mayer, A. M., E, Harel and R. B. Shaul. 1965. Assay of Catechol oxidase a critical comparision of methods. Phytochemistry. 5:783-789.

[15] Almeida, N. F. J., and De. 2001. Fungicide treatment of corn seeds against soilborn fungi and the control of *Fusarium* associated with seeds. Rev. Plant pathol. 80:163.

[16] Gauperin, M., Graf, S and D. Kenigsbuch. 2003. Seed treatment prevents vertical transmission of *Fusarium moniliforme* making a significant contribution to disease control. Phytoparasitica. 31:344-352.

[17] Liat, O., E. Smadar., C. David and S. Amir. 2002. Early Events in the *Fusarium verticillioides*-Maize Interaction Characterized by Using a Green Fluorescent Protein-Expressing Transgenic Isolate. Department of Plant Sciences, Tel Aviv University.