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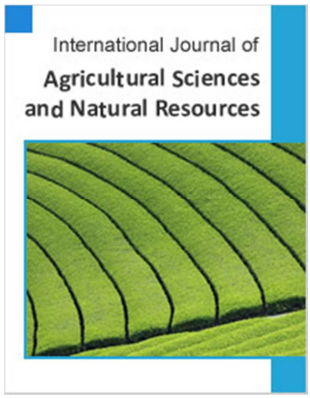

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Identification of Fungi Allied with the Rot of Unripe Pepper *(Capsicum annuum)* Grown in Wukari, Taraba State, Nigeria

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Abstract
The research investigation of fungi associated with the rot of unripe pepper *Capsicum annuum* was carried out using fifty (50) of unripe pepper *Capsicum annuum* samples from four (4) different farm sites spread across Wukari town. Isolation of the isolates was done using standard microbiological techniques with Potato Dextrose Agar (PDA) and incubating at room temperature (28 ± 2°C) for up to 14 days. Five (5) different fungi were isolated from the rotted fruits, which were *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus nidulans*, *Penicillium species* and *Fusarium oxysporum*. In terms of number of isolates and percentage frequency of isolates, *Aspergillus niger* 7 (25.9%), *Aspergillus fumigatus* 5 (18.5%), *Aspergillus nidulans* 4 (14.8%), *Penicillium species* 1 (3.7%) and *Fusarium oxysporum* 10 (37%). Among these isolates *Fusarium oxysporum* 10 (37%) had the highest frequency of occurrence while *Penicillium* species 1 (3.7%) had the lowest frequency of occurrence. *Fusarium oxysporum, Aspergillus niger, Aspergillus fumigatus* caused the soft rot of unripe pepper fruits after 48 hours incubation when pathogenicity test of the isolates was done using fresh and well sterilized unripe pepper *Capsicum annuum*. On the other hand, *Aspergillus nidulans and Penicillium species* did not cause soft rot of the unripe pepper. In conclusion, the immediate practical measure of reducing spoilage may be by educating the farmers and traders on proper handling methods of these fruits as the major factors noted in the course of this study to be responsible for the spoilage was due to wounds, cuts and bruises of the fruits. Each of the wounds served as an entrance for the various soft rot pathogens and associated microorganisms. Also, some fruits carry several destructive pathogens that manifest themselves in the storage thus leading to the reduction in the market value of the product. To this end, the control of fruit rot should include the use of chemicals in the field and after harvest as these will helps to maintain the quality of fruits during growth, harvest and storage. Also, use of resistant varieties in the propagation of the fruit can also help and the soil should be tested and treated if found to have any pathogen that could affect the fruit after planting. The appropriate application of these suggestions will greatly reduce the losses and provide a better supply of high quality and affordable products of *Capsicum annuum*.

1. Introduction

Pepper is one of the family of *Solanacese*, order *solanacese* and genus is *Capsicum*. The *Capsicum* are very old and are widely spread in the North America and the Eastern tropics except in the cooler parts [1]. In many countries today, they are the most important spice as in the case in Nigeria, where it is used in making hot sauces. The long period of cultivation has resulted in many varieties differing in habit, size, shape, color and pungency of the fruits [2]. Some authorities consider the capsicum to belong to two or three distinct spices *Capsicum annum* or *capsicum frutescence* [1]. The pepper fruits are of various types: Black Pepper (*Piper nigrum)*: it is of the family of piperceae. It is weak climbing or trailing shrub with adventitious roots. The fruits are small, one seeded berry- like drupes on ripening, and they are bright red or yellow. They require a hot humid climate and partial shade for growth. They are used as flavoring for soups and non- alcoholic beverages. They are also sold as peppercorns or in ground forms [3]. *Capsicum annuum* which is usually an annual or short-lived perennial herb up to 1.5m in height, a well-developed tap root, often with many lateral roots, the skin branched, erect or semi-prostrate, fleshy, often woody at the base, round or slightly angular, growth normally indeterminate, the leaves alternate, simple, ovate to lanceolate, margins entire, tip pointed, variable in size, up to 12cm long, 7.5cm wide. The flowers single, sepals, campanulate, sometimes expanding with fruit development, petals 5 to 6, white-green, up to 15mm in diameter, anthers 5 to 6, style with capitates stigma. The fruit are many-seeded berry, hollow, rectangular to long, with two or more locules, variable in colour and pungency, 1- 15cm long, 1-4cm in diameter. The seeds are flattened and kidney-shaped, 3-5mm in length, pale yellow, 1000 seeds weigh approximately 5.5g [4]. Many cultivated forms of *C. annuum* exist; most based morphological forms of the fruit such as the following: Long peppers: 20-30cm in length, cream, yellow or red tapering. Sweet, bell and paprika pepper: large, inflated thick-fleshed. Chilli or chili pepper: more than 9cm in length, narrow, pointed. Wrinkled peppers: less than 5cm in length, wrinkled. Cherry peppers: rounded, firm flesh, yellow, red or purple. Tabasco peppers: small, conical, and erect. Cluster peppers: fruits in clusters [4]. *Capsicum annuum* is widely distributed throughout most tropical and subtropical areas. The unripe fruit is green or purplish, ripening to red, orange, yellow, brown, or purplish. The pollination is through both self and cross pollination. The latter being about 16%. Anthesis takes place time after the flowers have opened. Flowers remain open for 2-3 days. The percentage fruit set is 40-50 percent [5]. The fruits have the mildest flavor with little of the pungent principle. They are eaten raw in salads and cooked in various ways; they are often stuffed with meat and are also picked [5]. *Capsicum* is also used industrially for the production of paste. 'Tomapep' is a paste made from a mixture of tomatoes and pepper used in soup preparation. It is used externally as a counter irritant and extensively used in such beverages as ginger ale because of its pungency [1]. It is also preserved in brine [4, 5]. Fruits and vegetables are susceptible to rot pathogens. Over the years, peppers have constituted a third of the total quantity of perishables in the domestic market. Sweet peppers are not well adapted to long time storage, for they are subjected to injuries at low temperature and at high temperature. Most workers who have conducted market surveys have found it convenient to recognize only three broad primary causes of fruit loss, these are: Physical or mechanical bruised injuries during post-harvest handling operations, like finger nail damage to the skin of the fruit or injuries from basket. Non-parasitic or physiological damage. Parasitic damage – diseases caused by fungal or bacteria pathogens [6]. Extent of damage due to these causes varies according to wide range of factors such as the cultivation, origin, growing environment and marketing condition. At both farmers and marketer's levels, a lack of knowledge of adequate post- harvest handling procedures from handling procedures from holding in the field and throughout the distribution network acts as a major constraint to the maintenance of fresh field quantity. The microorganisms that infect fruits are most aggressive when the host is nearing senescence. In this situation, pathogens may merely increase tissue deterioration which predisposes the tissue to further infection. Rots, either dry or soft are necrotic infections that may be localized or general on plants part [7]. [8] Gave a concise definition of rot as decay, a decomposition or disintegration of plant tissue. Rot diseases makes the plant product unfit for human consumption or as animal food, and where adequate storage facilities are lacking, losses can be great. The fruits when harvested by local farmers are piled into open baskets on top of one another or they are put into sacks or fiber bags. In either case, the fruit receives very little protection from injuries which occur extensively. The wounds readily become colonized by propagules of pathogens contained in fluids leaking from some already rotted fruits, coupled with changes due loss of water often occurs, such changes do not only affect the fruits but also predisposes them to microbial attack [9]. High water content in fruits and vegetables suitable temperature and high relative humidity also favours the germination of microbial spores which facilitates rapid disease development [2]. Temperature is the most powerful determinant factor of fresh produce deterioration rate. Also, genetic composition influences senescence in pepper fruit. According to [4], cultivars similar in various phenotypic respects can still differ substantially in their rates of senescence, susceptibility to disease and other characteristics. A number of microorganisms have been recognized as a major spoilage agent of the fruits of capsicum. It was found out that pathogenic and saprophytic fungi and bacteria were present on the plant and fruits of capsicum [8]. It was also discovered that soft rot of pepper is caused by *Rhizopus stolonizer* and *R. oryzae*. *Rhizopus* infected pepper fruits become covered with a fluffy mass of the fungus mycelia [10]. Dry rot of pepper is caused by *Aspergillus flavus*, *A.*

fumigatus, A. scleatus resulting in the collapse and twisting of the infected pepper fruits. Fruits infected with *A. flavus* and *A. fumigatus* look dry, wrinkled with obvious discoloration. Also, *Fusarium eguisei* and *Fusarium oxysporum* are also associated with pepper infection, while *oxysporum, rhizopus*, *oryzae* and *Fusarium oxysporum* has a frequency of 13.35% occurance on stored fruits [7, 8, 9]. The damage caused by these microorganisms and the resultant loss can all be influenced in various ways it could be due to poor marketing system, inadequate use of available technology during production, harvesting, selection, picking, transportation, storage, processing and distribution. Also, a lack of suitable infra-structures for the interchange and handling of agricultural products. A thick cuticle confers some degree of resistance to attack by invading microorganisms. If, however, the outer covering is ruptured, host resistance is consequently lost. Improved handling and packaging may reduce mechanical damage or wound or injury and is one of the important means of reducing spoilage of the pepper fruits [5, 8]. Also, the use of chemicals in the field and after harvest provides a means of maintaining quality during growth, harvesting and handling of the commodity. This study is therefore undertaken primarily to identify the fungi associated with the rot of unripe pepper *Capsicum annuum* grown in Wukari.

2. Materials and Methods

2.1. Sources of Sample and Media

A total of fifty (50) number of diseased *Capsicum annuum* fruits were collected for analysis from four different farms (north, south, east and west) in Wukari town. Samples were collected in clean polythene bag and taken to the laboratory for examination. Potato dextrose agar (PDA) medium was employed in the culturing and isolation process of associated pathogens.

2.2. Isolation of Associated Organisms and Identification of Isolates

Diseased fruits were surface sterilized with 70% alcohol and by rinsing in several changes of distilled water. Tissues of diseased fruits were plated on potato dextrose agar plates and the washing water for the first, fifth and tenth wash was also plated. Inoculated plates were incubated at room temperature (28±2°C). Purification of the isolates was done by subsequent sub culturing into fresh PDA media until pure cultures were isolated. Identification of isolates was carried out using cultural, morphological characteristics and viewed under the microscope for the presence or absence of cross walls/septa and or reproductive structures.

2.3. Pathogenicity Test

Pathogenicity test were carried out by inoculating healthy pepper fruits with the spores or hyphae of isolates. Fruits were carefully selected to avoid evident bruises and blemishes. They were surface sterilized with 70% alcohol and washed in several changes of sterile distilled water. Laceration were made on some of the healthy fruits and then inoculated with pure and mixed culture while some were inoculated without laceration. All inoculated fruits were incubated at room temperature, (28±2°C).

3. Results

The results of this study from the four (4) different farms in Wukari showed five (5) different fungi isolates from the unripe pepper fruits of *Capsicum annuum* and the percentage frequency of occurrence of isolates is shown in table 1. Table 2 show the morphology and cultural characteristics of isolates.

Table 1. *Percentage frequency of occurrence of Isolates.*

S/N	Isolates	Number of Isolates	Percentage of Isolates (x/y × 100)
1	*Aspergillus nidulans*	4	14.8
2	*Aspergillus niger*	7	25.9
3	*Aspergillus fumigatus*	5	18.5
4	*Penicillium species*	1	3.7
5	*Fusarium oxysporum*	10	37.0

Key
X=Number of isolates
Y=Total number of isolates

Table 2. *Morphology and cultural characteristics of isolates.*

S/N	Cultural characteristics	Morphology characteristics	Isolates
1	Grey to green	Growth is velvet-like and flaky with marked sporulation. The hyphae are septate while conidia are arranged in chains on top of prominent sterigmata	*Aspergillus fumigatus*
2	Black	The growth is cottony and highly sporulation. The hyphae are septate, conidia are unicellular, black and arranged in chains. It possesses a short and small globule vesicle.	*Aspergillus niger*
3	Light green	Its growth form is velvet-like and the fungus is highly sporulation. Its hyphae are septate and bear philaids on its sterigmata which are borne directly on the expanded vesicle.	*Aspergillus nidulans*
4	Bluish green	Growth form is powdery or velvet surface on the substratum. The conidiophores are long, erect, septate and highly branched at the tip to form phialides. Globose conidia are attached to the phialides terminating in a chain of conidia. The conidium is hyaline, one celled and smooth.	*Penicillium species*
5	Initially white and develops to pink with age	Growth form is wooly. Conidiophores are septate and branched. Two types of conidia are found, the macroconidia and microconidia. The macroconidia are long, septate, and sickle shape with 3-4 septas while the microconidia are small and ovoid.	*Fusarium oxysporum*

4. Discussion

The investigation revealed five (5) different fungi species isolated from unripe pepper- *Capsicum annuum* grown and sold in Wukari town. The fungi isolates are *Aspergillus niger, Aspergillus fumigatus, Aspergillus nidulans*, *Penicillium species and Fusarium oxysporum*. Out of these five (5) isolated, *Aspergillus niger* and *Fusarium species* had the highest occurrence which is in agreement with the work of [7]. Infection starts from any wounded part of the fruit. The first sign is the softening of the affected tissue which takes on a sunken cooked appearance and this also agreed with [6, 9] which shown that microorganisms are usually associated with any opening with sign of fluid that can easily support their growth. At the advance state of the disease, the mycelium is evident on the decaying tissue; the tissue becomes grayish in colour and later dense grayish mycelia that develops which produces conidia under moderately humid conditions [3]. Isolation from the fruit revealed *Aspergillus* species as the causal organisms. These species were found to be associated with the rotted fruits of pepper in the different samples [1, 4]. All fungi isolated are capable of growing on substrate with high moisture content [2]. Microorganisms usually do not affect substrates singly except under laboratory controlled conditions. And the infection of substrates by the invasion of other saprophytes that may be harmful to man [11]. The ingestion of any infected product should therefore be discouraged. The study also reveals that bruises or injuries to fruits make them more susceptible to infection which aligned with previous work of [4, 6, 8, 10]. The healthy fruits are only susceptible to infection if the storage conditions are more favourable to the pathogens [11] From this study also, it was found that *Fusarium oxysporum* was involved in the soft rot of the unripe pepper. This gave a different dimension to other available literatures where it was only involved in dry rot of pepper.

5. Conclusion

The immediate practical measure of reducing spoilage may be by educating the farmers and traders on proper handling methods of these fruits as earlier stated. The major factors responsible for the spoilage were due to scratches, wounds, cuts and bruises of the fruits, which served as an entrance to the various soft rot pathogens and associated microorganisms. Also, some fruits carry several destructive pathogens that manifest themselves in the storage thus leading to the reduction in the market value of the product. The control of fruit rot includes the use of chemicals in the field and after harvest which will helps to maintain the quality of fruits during growth and harvest. The use of resistant varieties in the propagation of the fruit can also help and the soil should be tested and treated if found to have any pathogen that could affect the *Capsicum annuum* fruit after planting. The application of these recommendations will greatly reduce the losses and provide a better supply of high quality *Capsicum annuum* product at affordable prices in our today market.

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