International Research Journal of Natural Sciences
Vol. 2, No. 1, pp. 40-51, March 2014
Published by European Centre for Research Training and Development UK (www.ea-journals.org)

DIFFERENTIAL RESPONSE OF *Solanum tuberosum* L. AND *Ipomea batatas* L. TO THREE ROT PATHOGENS.

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ABSTRACT: *Studies on comparative response of two potato species (Irish potato - Solanum tuberosum and Sweet potato - Ipomea batatas) to three tuber rot pathogens, Botryodiplodia theobromae, Rhizopus stolonifer and Penicillium expansum. were carried out in Calabar, Nigeria. Results showed that within four weeks of the experiment, these pathogens consistently caused starch grain depletion from the tissues of the two potato species studied. The pattern of colonization differed between them. In the first week of incubation, B. theobromae caused a reduction of the size of the grains from 10mm to 5mm in S. tuberosum before digesting them. Less than 10% of the starch was lost; while S. stolonifer and P. expansum individually recorded 20% starch grain depletion within same period. In I. batatas, reduction in the size of the starch grains was only evident in R. stolonifer – treated tubers, with negligible loss recorded within this period (first week of incubation). By the 3rd week of incubation, R. stolonifer and B. theobromae had recorded total starch depletion in I. batatas tuber tissues. All the treatments except for P. expansum- treated I. batatas tissues recorded zero starch presence, extensive tissue necrosis and massive collapse of cell wall by the end of the 4th week of incubation. Differences in the sizes of starch grains were also observed in healthy tissues of the two potato species used in this study. The sizes of starch grains in I. batatas were smaller and more tightly packed than those found in S. tuberosum.*

KEYWORDS: Potato Species Rot Pathogens, Starch Grain Depletion.

INTRODUCTION

Potato is an annual or perennial plant with edible underground tubers. About 50 genera and more than 1000 species are known. Of these, it is only sweet potato and lrish potato that are widely cultivated and consumed (FAO, 1995). Irish potato is reported as one of the eight leading staple food crops in the

world (FAO, 2008). Both species are known to be eaten boiled roasted, baked or fried. However, potato exhibit poor texture when cooked and are often processed into a very wide range of food products, especially, if the sugar content is within 2% (Cumming, 1996). Such processed products include, canned whole potatoes, frozen French fries or chips, powder or potato salad (FAO, 2005). The crop has also been employed in the production of large – grained starch which is one of the raw materials for paper and textile industries. It is also useful in the manufacture of alcohol (FAO, 1998). Potato flour is used in the preparation of certain types of bread, pastries, cakes and biscuits.

The crop is highly rich in nutrient elements. A 100g edible portion of sweet and Irish potatoes yield 360kj and 321Kj of energy, 20.1g and 19.1g carbohydrate, 12.7g and 15g starch, 3.0g and 2.2g dietary fibre, 0.1g and 0.1g fat, 1.6g and 2.0g protein, 30.0mg and 12mg calcium, 0.6mg and 1.8mg iron, 25.0mg and 23mg magnesium, potassium 337mg and 421mg, thiamine 0.1mg and 0.08mg, riboflavin 0.1mg and 0.03mg, phosphorus 47.0mg and 57mg, sodium 55mg and 6mg, zinc 0.3mg, folate 11Ng, pantothenic Acid 0.8mg, Niacin (B3) 0.61mg and 1.1mg, and 79.8% and 75g Water in Sweet and Irish potato respectively. About 2.4mg vitamin C is reported in sweet potato and 0.25mg for Vitamin B6 (FAO, 1998).

In spite of its numerous uses, many insect pests and microorganisms attack potatoes both in the field and in storage. The susceptibility of potatoes to microbial attacks in storage is a function of their high moisture content (70-80 %). The major storage disease of potato is rot. The organisms implicated in rot diseases include bacteria, nematode and fungi. Of these, fungi are the most virulent agents and the most common cause of rot diseases (Isaac, 1992). Among the numerous fungal agents of rot diseases is *Botryodiplodia theobromae,* the causal agent of rot of potatoes (IITA, 1993). Losses due to rot diseases have been reported to be as high as 30-60 % during the course of 3-6 months storage period (Arinze, 2005). Losses are in the form of reduction in the quality of the tubers through breakdown of tissues resulting in anatomical aberrations and depletion of the nutrient component such as protein, lipids and the major one – starch (Markson *et al.,* 2010a; Arinze, 2005). Information on the gravity of damage of tissues and the amount of nutrients (starch) lost within a set period of infection is scanty. Information on the anatomical aberrations and starch depletion in sweet and Irish potatoes is here presented.

MATERIALS AND METHODS

Sample collection and pathogen identification

Symptomatic and asymptomatic tubers of sweet and Irish potato (*Ipomea batatas* and *Solanum tuberosum*) were obtained from open market stalls in three markets in Calabar urban, Cross River State, Nigeria. The markets were Akim, Marian and Watt. Tissues (about 5mm in diameter) from the symptomatic and asymptomatic sweet and Irish potato tubers were removed using a 5mm diameter cork borer following surface sterilization with 70% ethanol for 10 s. the tubers were blotted dry with sterile paper towel, and plated onto chloramphenicol-amended Potato Dextrose Agar (PDA). After three days of incubation at 28^0C, microbial growth was assessed by microscopy. Cultures that were

suspected to be *Botryodiplodia theobromae* based on morphological characteristics were transferred to new PDA-containing plates, from where axenic cultures were generated (Markson *et al*., 2010a). Cultures were thereafter identified as *B. theobromae* based on morphological characteristics described in the 1987 illustrated genera of fungi by Barnett and Hunter and with literature on identification of pathogenic fungi by Rossman *et a*l, (1997). Confirmation was made by comparing with a culture identified by International Mycological Institute, Egham, UK. (IMI 347961).

Koch's postulates and pathogenicity test

To confirm the pathogenicity of the isolates from the two potato tubers, axenic cultures of each of the isolates were used to inoculate the tubers of each species of the potatoes with 5- mm-diameter mycelial agar plugs from a 4-day-old culture. On appearance of symptoms, the tissues at the margins of the healthy and diseased parts were surface-sterilized, excised and plated onto PDA for incubation at 28^{o}C for four days. At the end of this period, morphological characteristics and growth patterns observed in each case were compared with the ones of the original isolates.

Anatomical studies of the potato tubers

The method used was the modification of the method described by Arinze *et. al.* (1985) to determine the mode of entry by the pathogens into the host tissues. Tubers of the sweet and Irish potatoes were peeled and surface-sterilized by dipping them in calcium hypochlorite (3 % available chloride) for 3mins. Rinsed with several changes of distilled water and allowed to dry at room temperature (28^{0}C). With a sterilized kitchen knife, about 1 cm thick slices were obtained from the tubers in each case. These were placed in sterilized glass Petri dishes and inoculated with spore (conidia) suspension. The spore (conidia) load was estimated using haemacytometer.

The inoculum load was determined using the formula:

$$\text{Spore Load} = \frac{N \times v}{V}$$

Where , N = mean number of spores counted in the chosen square (total amount of spores counted divided by the number of squares).

V = volume of the mounting fluid (sterile distilled water)

v = volume of the mounting solution between the cover glass and above square counted (area of square × depth of the chamber).

A spore (conidia) load of 5.0×10^{4}/ml of sterile distilled water were used in all the experiments except when otherwise stated. Inoculated slices of tubers in the Petri dishes were incubated at 30^{0} C and then sectioned after a week, then at interval of every other week for a period of 5 weeks. Uninoculated slices (control) were similarly sectioned.

Preparation of tissue sections of inoculated and uninoculated yam.

Sectioning was done by first fixing the inoculated and the uninoculated slices of the sweet and Irish potato tissues separately with F.A.A (1:1:18) for 24 – 28 hrs then rinsed with distilled water. Tissue sectioning was done at 10 mm depth using Reichert Rotary microtome. The sections were then stained with safranin for 2 – 3 minutes and dehydrated using pure xylene. These dehydrated sections were then mounted in Canada balsam on a glass slide. The slide was dried over a hot plate at 35 – 40^0C. Photomicrographs of the prepared slides were taken using Leitz Weitzler Ortholux microscope fitted with a Vivitar – V335 camera (Markson *et al*., 2010a).

RESULTS AND DISCUSSION

Sample collection and identification of isolates

Following isolations from rotting tissues of sweet and Irish potatoes, three organisms (*Botryodiplodia theobromae*, *Rhizopus stolonifer* and *Penicillium expansum*) were obtained. Fungal colonies that emerged on the culture medium (PDA) were identified. On PDA, colonies of *B. theobromae* were initially white, fluffy and feathery, becoming grey and eventually black. The growth was radial in pattern from the centre of the plate outwards. *R. stolonifer* exhibited profused growth with their whitish thread-like mycelia dotted with grey coloured sporangiospores after three days of growth. The colony of *P. expansum* was greenish in colour and appeared crust-like on the growth medium. Literature on identification of pathogenic fungi (Rossman *et al*., 1997) and illustrations by Barnett and Hunter (1995) corroborate these observations and the appearance of *B. theobromae* fitted the description of *Botryodiplodia* Pat. (=*Lasiodiplodi theobromae* (Pat.) Griff and Maubl.) as presented by Marley (1998). The true identity of each of these fungi was confirmed by comparing their cultures with those identified by International Mycological Institute, Egham, UK.

Koch's postulates and pathogenicity test

The *B. theobromae*, *R. stolonifer* and *P. expansum* isolates were pathogenic on the potato (sweet and Irish) tubers used for each pathogen for the test. Symptoms of decay (rot) caused by *B. theobromae* was seen as dry black rot. *R. stolonifer* produced soft rot symptoms and *P. expansum* caused dry rot on the potato tubers tested. On re-isolation, the three isolates exhibited similar pattern of growth as observed in the original isolates.

Anatomical studies of the potato tubers

The value of tubers is in the quantity and quality of their nutritional content. The nutrient in high amounts in the tubers is starch. The impact of attacks by *B. theobromae*, *R. stolonifer* and *P. expansum* on the starch content and tissue integrity of sweet and Irish potatoes was investigated. Results showed that in the first one week of incubation, *B. theobromae* and *P. expansum* caused reduction in the size of the starch grains by ½ (from 10mm to below 5mm) in *S. tuberosum* (Figure 1c and e). During this period, about 10% of starch grains were removed as against a 20% reduction obtained with each of *P.*

expansum and R. stolonifer. In *I. batata*s, reduction in the size of the starch grains was only evident in *R. stolonifer* –treated tubers (Figure 1h), with negligible starch loss. Other treatments also recorded minimal cellular alterations (B3 and B4) within this period. The minimal damage evident in the affected tissues may have resulted only from polygalacturonase which is the cell wall degrading enzyme reported to be the first to be secreted by rot pathogens (including *B. theobromae.*) during tissue invasion (Cooper, 1987). This corroborates the findings of Markson *et al.,* (2010b) who reported slight loss of starch grains from tissues of white yam infected with *Botryodiplodia theobromae* in the first seven days of incubation. Within this period, they also reported minimal maceration of the cell walls of the affected tissues. Uninoculated samples were not affected. They correlated the disappearance of starch with the depletion of carbohydrates from the tissues and attributed this to the ability of the pathogen to secrete carbohydrate – degrading enzymes.

In our findings, *B. theobromae* was still very slow in starch grain removal and general tissue destruction in *S. tuberosum* even after two weeks of incubation compared with *R. stolonifer* and *P. expansum.* This observation differed with the submissions by Markson *et al*., (2010a) that the rate of starch depletion was more in the first three weeks of incubation. Though we recorded a more accelerated rate of starch depletion by *B. theobromae* in the 3rd week, the delay observed in the first two weeks of this study may be due to the difference in the physicochemical constituents of the tubers used in these two separate experiments. Increased loss of starch grains from the potato tissues in the 3rd week may have resulted not only from the activity of cell wall degrading enzymes but also from increased rate of respiration by the affected tissues in response to invasion by the pathogen (Prasad *et al.,* 1989). In *I. batatas, B. theobromae* caused cell shrinkage (cell size reduction) accompanied with minimal depletion of starch, while *P. expansum* recorded total cell wall collapse, necrosis and massive starch grain removal. *R. stolonifer* caused extensive cell necrosis and expansive cell wall maceration. The result for the second week indicated that tissue damage was greater in *I. batatas* than in *S. tuberosum* with increasing weeks of infection (Figure 2a -2h). The ease of colonization of the *I. batatas* and preference by the test pathogens may be a function of the difference in tissue texture of the tubers which is usually conferred by the sizes and the patterns of arrangement of the starch grains in the tissues of the tubers (FAO, 1998).

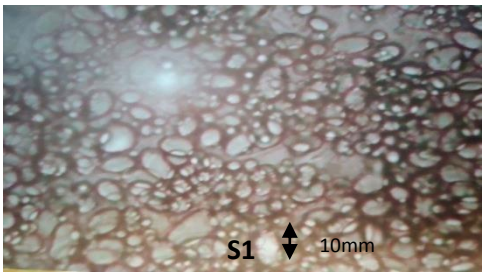
After three weeks of incubation, tissues of *I. batatas* infected with *R. stolonifer,* recorded total depletion of starch grains. Similar result was recorded in *B. theobromae* –treated tissues (Figure 3h and d). However, pockets of starch grains were still visible in tissues treated to infective propagules of *P. expansum* after three weeks of incubation. Tissues of *S. tuberosum* infected with *R. stolonifer* revealed massive erosion of cell wall boundaries and near total depletion of starch grains when compared with *B. theobromae- and P. expansum*-incited tissues (Figure 3c and e). All the treatments except for *P. expansum*- treated *I. batatas* tissues recorded zero starch presence by the end of the 4th week of incubation. There was, however, a slight reduction in the starch grains in the control treatment (Figures 3a and b) at the end of the experiment (Figure 4a-h). The gradual and consistent reduction in the starch

grains evident in the control experiment is probably due to cellular respiration in the fresh tissues of potato resulting in the oxidation of starch to water, carbon dioxide and heat hence reducing the dry matter (FAO, 1998).

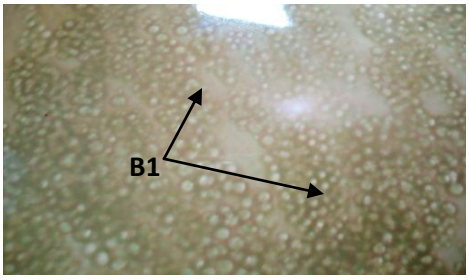
CONCLUSION

The findings from this research revealed that potato tubers attacked by these test fungi beyond two weeks are not fit for consumption as they will no longer provide the major food nutrient (starch) they were supposed to have supplied to the body, due to the activity these pathogens. This points to the fact that it is not enough to plant and harvest high yielding potato tubers but it is pertinent and most important to protect these crop produce in storage to circumvent post harvest losses which, most of the time, is higher than losses recorded in the field (Arinze, 2005). Hence to maintain the quality of their harvested produce, farmers should take a more serious look at storage than is generally done now, especially in Africa. The level of post harvest losses recorded in Africa can be greatly reduced if the issue of storage is given equal attention as is allotted yield improvement.

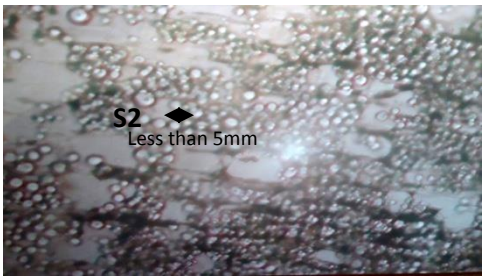
The panacea to food security is not just crop production but rather crop protection. A crop well produced and less protected is a crop lost. And a crop lost is worse than a crop not cultivated as the labour cost, time, energy and the land cultivated to the crop at that time space becomes a waste. The quality of any crop depends on the level of nutritional content of such a crop (Markson *et al.,* 2010b). The successful preservation of these tubers from pathogenic attacks is inextricably anchored on the knowledge of the routes through which such losses could be incurred, as this will form the basis for adopting effective management strategies. Effective disease management strategy is proactive and prophylactic rather than curative. Farmers should update their knowledge on new and more effective but cost- friendly storage methods through attending seminars and workshops in this area.
It is time to direct the quest for food security on protecting the food we have successfully produced than continuously producing plenty of food that is finally lost because it was not sufficiently protected.

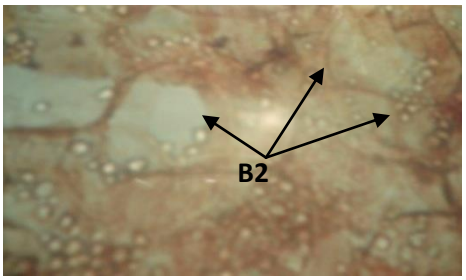


a) *Solanum tuberosum* Control (Uninfected)

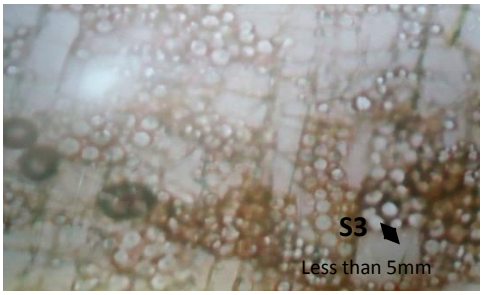


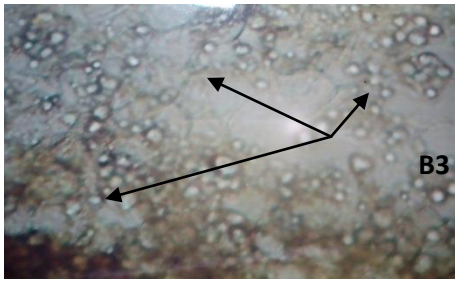
b) *Ipomea batatas* Control (Uninfected)



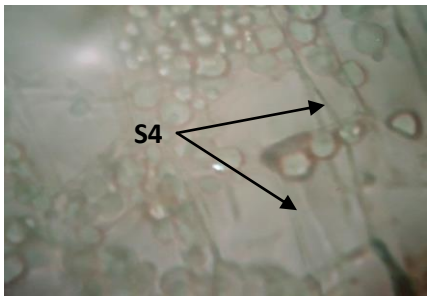


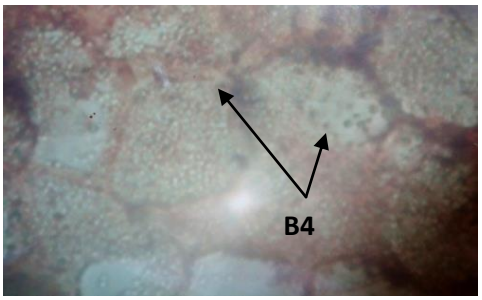
B. theobromae on: c) *Solanum tuberosum* d) *Ipomea batatas*





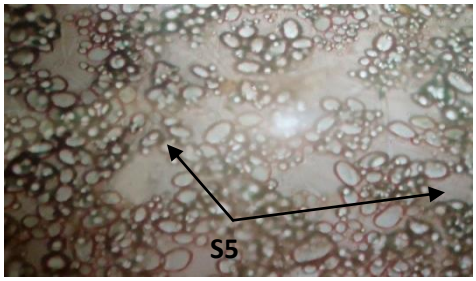
P. expansum on: e) *Solanum tuberosum* f) *Ipomea batatas*

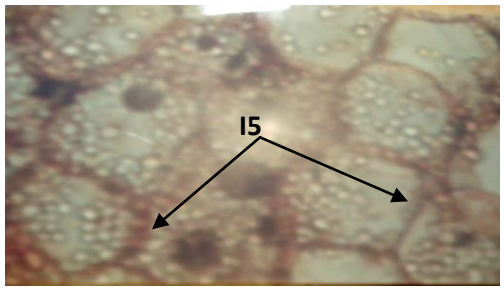




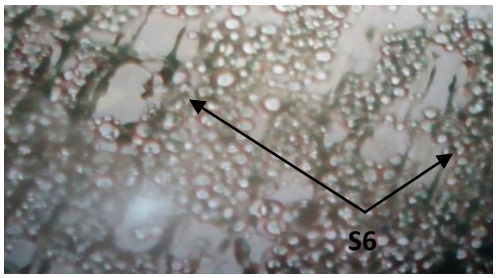
R. stolonifer on: g) *Solanum tuberosum* h) *Ipomea batatas*

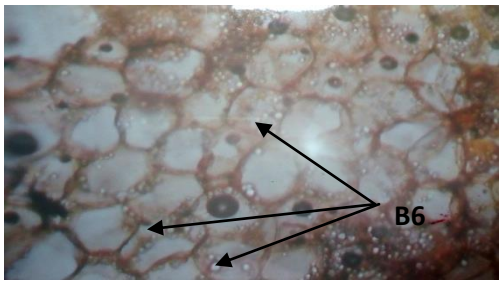
Figure 1(a) Fresh uninoculated tissues of *S. tuberosum* and (b) *I. batatas* revealing a loosely packed arrangement of starch grains (X250). (c) reduction in size of starch grains from 10mm to 5mm (**S2**) by *B. theobromae* and *P. expansum* one week after infection. (d-h) slight depletion of starch grains of *I. batatas* and *S. tuberosum* by *P. expansum* and *R. stolonifer* within 1 week of incubation.



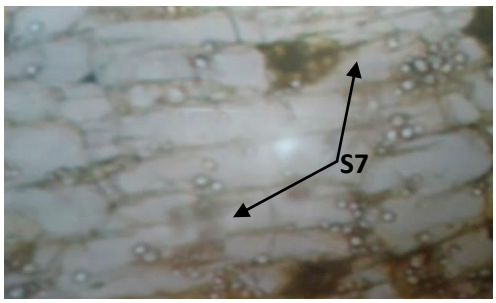


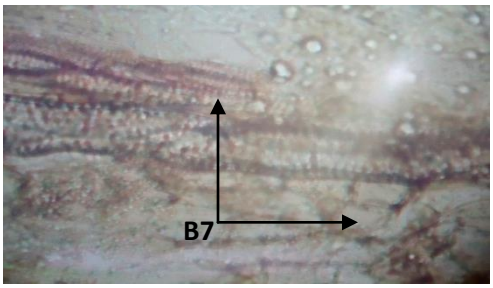
a) *Solanum tuberosum* Control (Uninfected) b) *Ipomea batatas* Control (Uninfected)



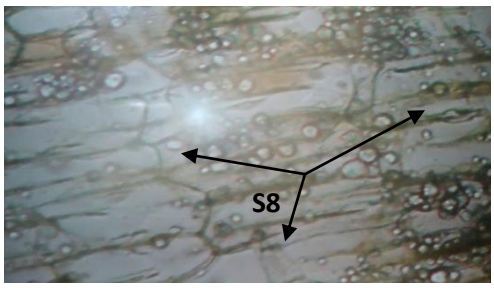


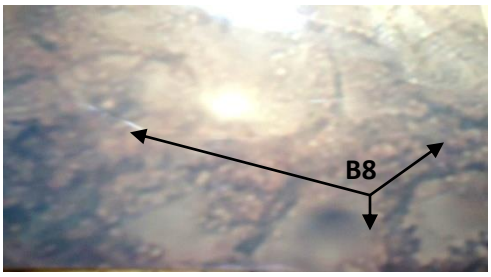
B. theobromae on: c) *Solanum tuberosum* d) *Ipomea batatas*





P. expansum on: e) *Solanum tuberosum* f) *Ipomea batatas*

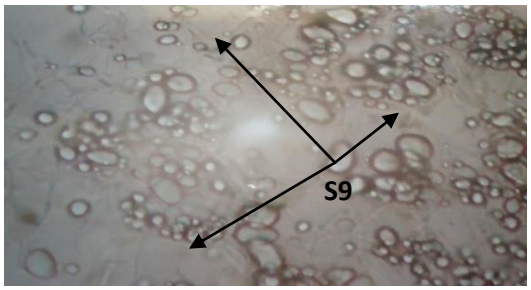


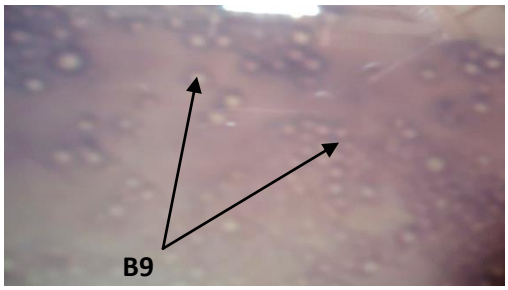


R. stolonifer on: g) *Solanum tuberosum* h) *Ipomea batatas*

Figure 2: (a-b) Uninoculated control showing very slight reduction in starch grains. (c-d) empty cavities evident in *B. theobromae-treated S. tuberosum* tissues (S6); cell shrinkage and cellular

necrosis (B6) shown in cells of *I. batatas* attacked by the same pathogen.(e-f) starch depletion, cell wall damage (S7) on *S. tuberosum* and total cell wall collapse (B7) in tissues of *I. batatas* incited by *P. expansum,* (g-h) cell wall erosion, cell necrosis and evacuation of starch grains in *S. tuberosum;* cells no starch grains, tissue necrosis and massive disintegration of cellular structures in *I. batatas* caused by *S. stolonifer.*





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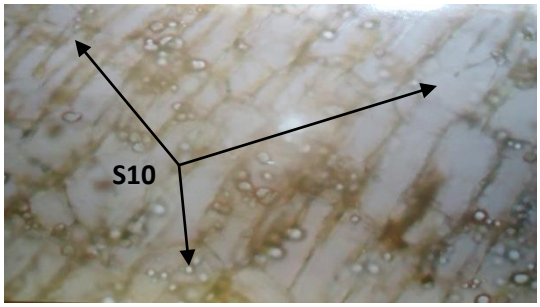
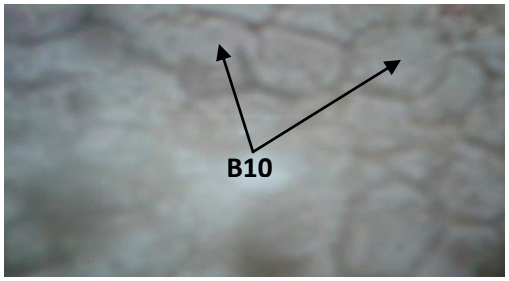
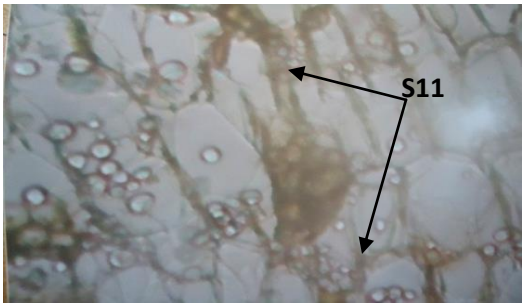
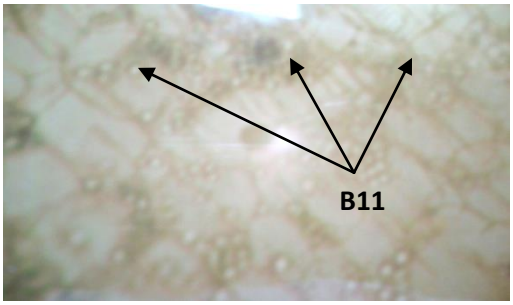


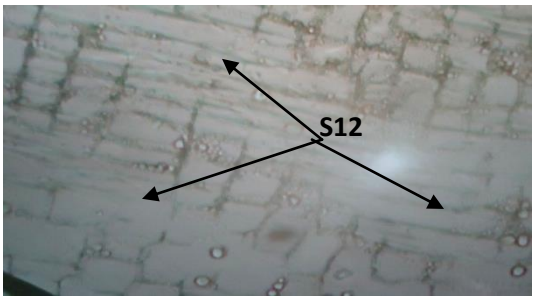


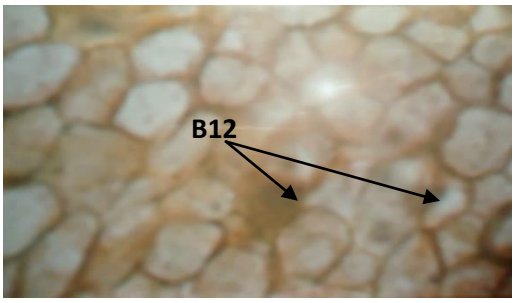
B. theobromae on: c) *Solanum tuberosum* d) *Ipomea batatas*





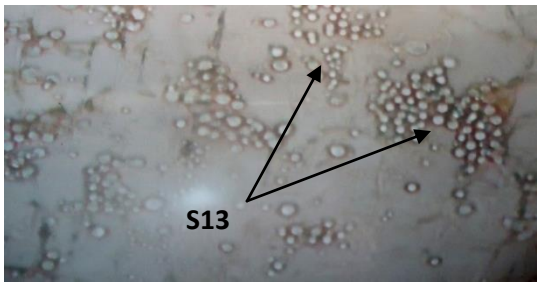
P. expansum on: e) *Solanum tuberosum* f) *Ipomea batatas*

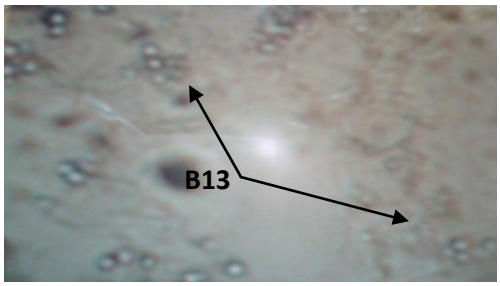




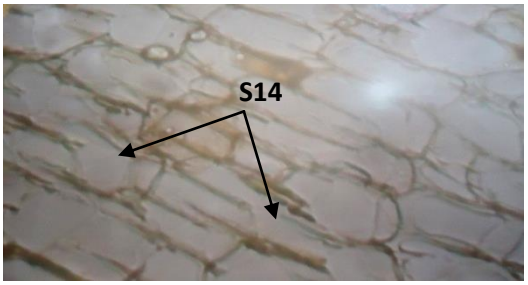
R. stolonifer on: g) *Solanum tuberosum* h) *Ipomea batatas*

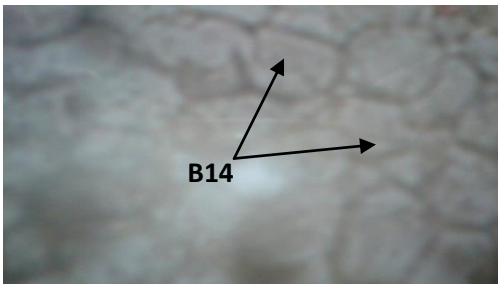
Figure 3: (a-b) showing gradual reduction in starch grains in *S. tuberosum and I. batatas* uninoculated controls. (c-d) cell wall collapse and tissue necrosis (S10) in *S. tuberosum;* near total starch grain evacuation and tissue maceration (B10) in *I. batatas* incited by *B. theobromae.* (e-f) necrosis, empty cells (S11) in tissues of *S. tuberosum;* total alteration (B11) in cellular structures of *I. batatas* caused by *P. expansum.* (g-h) near total erosion of cell wall boundaries (S12); total starch depletion and tissue necrosis (H3) incited by *R. stolonifer*.



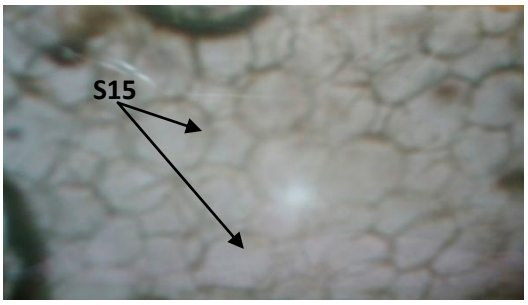


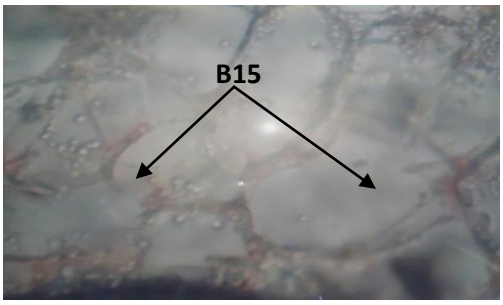
a) *Solanum tuberosum* Control (Uninfected) b) *Ipomea batatas* Control (Uninfected)



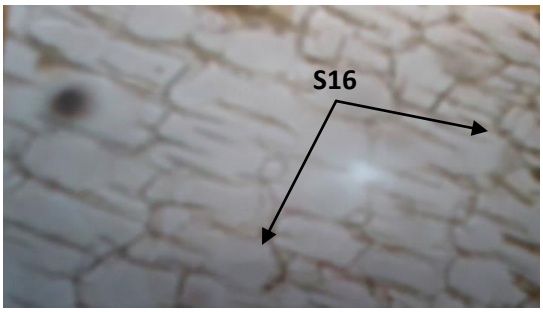


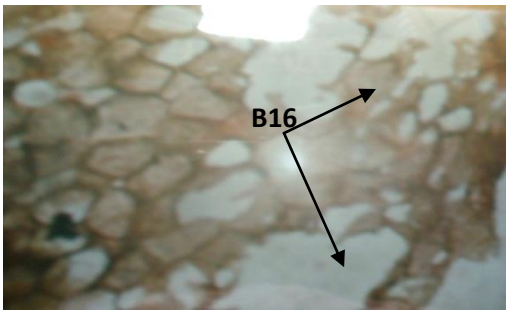
B. theobromae on: c) *Solanum tuberosum* d) *Ipomea batatas*





P. expansum on: e) *Solanum tuberosum* f) *Ipomea batatas*





R. stolonifer on: g) *Solanum tuberosum* h) *Ipomea batatas*

figure 4: (a-b) More starch grains lost from 4 –week old uninoculated tissues of *S. tuberosum* (S13) and *I. batatas* (B13). (c-d) empty cells and necrotic tissues of *S. tuberosum and I. batatas* caused by *B. theobromae.* (e-f) 4 - week old tissues of *S. tuberosum and I. batatas* attacked by *P. expansum* showing structural aberrations, necrosis and zero starch presence (S15); erosion of more cell wall boundaries leading to coalescing of many cells to form bigger cells with empty lumen (g-h) complete depletion of starch grains and total collapse of cellular structures of the potato tubers caused by R. stolonifer within 4 weeks of attacks

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