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The Spectrum of Infections by *Fusarium* Species on *Codiaeum Variegatum* (L.) Blume Cultivars as Influenced by Fructose Specific Lectin

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Abstract This study sought to have an insight into the mechanism of action of diseases responsible for the susceptible *Codiaeum variegatum* (garden croton) leaves induced by *Fusarium* species and the clinical importance of the disease resistant types. The plants were obtained from the Babcock University Germplasm Repository. Therefore, phytopathogenic *Fusarium* species were isolated from three diseased susceptible cultivars of *C. variegatum* (*ovalifolium*, *royal-like* and *punctatum*). Accordingly, lectin was isolated, purified and characterized from the leaves of a resistant garden croton cultivar (*royal*), and further evaluated for antifungal activity using isolates of *Fusarium lateritium* and *F. semitectum* obtained from the three diseased cultivars. The heamagglutinating activity of the purified lectin was non selective to type of blood group (A, B, AB and O) and was inhibited by fructose, sialic acid, and copper sulphate, but was enhanced by galactose, calcium chloride, sodium chloride and magnesium chloride. Optimum heamagglutinating activity of the lectin was achieved at 30–40℃ and pH 5.0–6.0. The lectin exhibited antifungal activity against the two *Fusarium* species in a non-concentration dependent manner. It is therefore concluded that *Fusarium* species are major phytopathogens of the garden croton plant and their spectrum of pathogenicity is dependent on the presence or absence of lectins. In addition, the resistant cultivar of *C. variegatum* (cv. royal) used in this study may be a suitable candidate for the prevention and treatment of fungal infections.

Keywords *Codiaeum variegatum*, *Fusarium*, Fructose, Galactose, Lectin, Systemic Acquired Resistance, Phytoallexins, Signal Transduction Pathway

1. Introduction

Lectins are carbohydrate-binding proteins that bind reversibly and possess the ability to agglutinate cells or precipitate polysaccharides and glyco-conjugates. They are widely distributed in animals, plants and microorganisms and have attracted great interest due to their various biological activities, such as cell agglutination, anti-tumor, immuno-modulatory, antifungal, antiviral and anti insect activities(1-2). Recent studies in glycobiology have emphasized lectins as the prime tools for cell-to-cell recognition in interactions involving numerous pathogens such as viruses, fungi, bacteria and the pluricellular parasites. The lectins recognize and bind to the oligosaccharides exposed by target cells and tissues thereby leading to the establishment of an infection (3). Conversely, the pathogen surfaces bear a large number of oligosaccharides that may be bound by specific lectins which can modulate the host infection (4-5).

These carbohydrates may be covalently bound, as in glycosylated teichoic acids to peptidoglycan, or non-covalently bound, as in capsular polysaccharides. Every surface-exposed carbohydrate is a potential lectin-reactive site. The ability of lectins to selectively form complexes with microbial glyco-conjugates makes them useful investigative tools for host-pathogen interaction. In the natural environment, microorganisms interact with each other to maintain their growth, development and stability within ecosystem. The interaction process can only be revealed by understanding the fundamentals of ecological relationships of diverse microbial population including pathogens and antagonists. In many instances, physical attachment of microorganisms is mediated by specific compatible macromolecules. The specific interaction between microbial populations may play a key role for successful establishment, persistence and colonization (6 –7). The role of cell surface macromolecules, potential ligands and receptors of cell

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surface agglutinins or lectins as recognition factor during attachment of two interacting partners, are well known (8-9).

Previous studies on the *Codiaeum variegatum* cultivars in the Germplasm Repository of Babcock University have associated some *Fusarium* species with the increased disease conditions of this ornamental plant (10- 11). However, no study has been carried out to investigate the mechanism of disease occurrence in cultivars members of the plant. In the present study, lectin purified from a disease resistant croton cultivar in the germplasm repository was partially characterized and evaluated for antifungal activity using two *Fusarium* species isolated from three other diseased susceptible cultivars of the garden croton ornamental plant.

2. Materials and Methods

2.1. Collection of Plant Materials

The leaves (diseased and non diseased) of four *Codiaeum variegatum* (garden croton) cultivars (*C. variegatum cv. royal*, *C. variegatum cv. ovalifolium*, *C. variegatum cv. royal-like* and *C. variegatum cv. punctatum*) (Fig. 1) were collected from the horticultural garden of Babcock University Germplasm Repository (BUGR), Ilishan-Remo, Nigeria. Field botanical characterization and identification of the cultivars was carried out by one of the authors who is a Plant Scientist and Biotechnologist in charge of the BUGR. *Codiaeum variegatum cv. royal* was collected as the resistant cultivar while *C. variegatum cv. ovalifolium*, *C. variegatum cv. royal-like* and *C. variegatum cv. punctatum* were obtained as the susceptible cultivars. The cultivars were placed in clean polythene bags and transported to the Microbiology laboratory of Babcock University for further analysis.

2.2. Isolation and Characterization of Leaf-Borne *Fusarium* in Garden Croton Cultivars

Leaf-borne *Fusarium* was isolated according to the method described by Dubey & Maheshwari (2006). Diseased leaves of each cultivar of garden croton were surface sterilized for 3 minutes in 2% sodium hypochlorite. The sterilized leaves were rinsed in two changes of sterile distilled water and blotted dry in two folds of Whatman No. 1 filter paper. Two 3mm discs each were obtained from the visibly infected and uninfected regions of the leaves of a cultivar, and plated out on peptone-pentachloronitrobenzene agar (PPA), a semi selective medium for *Fusarium* (13). Each set of two discs from the infected region was placed along the diameter line of the PPA plate at 3cm distance from each other. This was repeated for the discs from the uninfected region of the same cultivar. The set up was replicated thrice for each cultivar and all the inoculated plates were incubated for 3 days under fluorescent lights on a 12 h day/ night schedule at 22–24℃.

After 3 days, morphologically distinct colonies of *Fusarium* species that emerged from the leaf discs were transferred to ¼ strength potato dextrose agar (PDA) and incubated for 5 days as described above. Single spore was started for each isolate by micromanipulation on 2% water agar and the plates were incubated overnight at 22–24℃. The germlings were sub cultured from water agar and maintained on a modified Czapek's-Dox complete medium (CM). This was stored at 4℃ prior to identification. Each isolate was further sub cultured from CM onto carnation leaf agar (CLA for examination of sporodochia and uniform macro conidia under the Olympus BX51 Digital Microscopy, Olympus Optical Co., LTD, Japan) and full strength PDA (for pigmentation and colony morphology evaluation). The plates were incubated as above for 10–14 days. Morphological identification of the *Fusarium* species was performed as described by Leslie & Summerell (2006).

2.3. Pathogenicity Testing of *Fusarium* Isolates on Garden Croton Leaves

The pathogenicity of each *Fusarium* isolate obtained from the diseased parts of the croton leaves was tested by adopting the Koch's postulate as modified and reported by Dubey & Maheshwari (2006). About 20 young uninfected leaves were obtained from the apical parts of each cultivar and surface sterilized for 3 minutes in 2% sodium hypochlorite. The sterilized leaves were rinsed in two changes of sterile distilled water and blotted dry in two folds of Whatman No. 1 filter paper. Mild abrasions along the veins of each young uninfected leaf were induced using a sterile toothpick. Thereafter 0.1ml spore suspension containing 10^6 spores/mL of each corresponding *Fusarium* isolate was spread over the abrasions using a sterile cotton bud. Each inoculated leaf was placed on a moistened filter paper in a Petri dish which has been under-layed with cotton wool. All Petri dishes were incubated at ambient temperature for 5 days and the leaves were observed daily for symptoms of the disease on the inoculated part of the leaves. The diseased parts of the inoculated leaves were obtained, surface sterilized and plated on to ¼ strength potato dextrose agar (PDA). After a 5-day incubation period at 22–24℃ the associated *Fusarium* isolates were identified macroscopically and microscopically following the procedures reported earlier.

2.4. Blood Samples

Five milliliter each of 10 human blood samples of different blood groups (A, B, AB and O) was collected from Babcock University Medical Laboratory, Ilishan-Remo, Nigeria. The blood samples were collected in heparinised bottles containing (ethylene diamine tetra acetate, EDTA) to prevent coagulation and kept at 4℃ prior to analysis.

The blood samples were treated as described by Lis *et al.* (1994). Briefly, 5mL of each sample was centrifuged at 1500 × g for 5 minutes at ambient temperature. The red blood cells obtained were then washed by centrifugation at 1500 × g for 5 minutes at ambient temperature with 0.01M phosphate-buffered saline (PBS, pH 7.2). This was repeated

twice and the resulting cells were mixed with 3% formaldehyde in EDTA bottle and allowed to stir gently overnight prior to further centrifugation at 1500 × g for 5 minutes. The centrifuged blood cells were washed again as stated earlier, three times with 0.01M PBS (pH 7.2) and the cells were collected into a stopped bottle. About 76.8mL of 0.01M PBS was added to dilute the concentration of the cell. This concentration was then stored at 4℃ prior to further analysis.

2.5. Isolation and Purification of Lectin from *C. Variegatum* Leaves

Lectin was isolated and partially purified from *C. variegatum cv. royal* according to the procedures described by Awoyinka & Dada (2011) with slight modification. The leaves were pulverized and defatted using chloroform-acetone in ratio 2:1 thereafter the defatted samples were dissolved in distilled water (1:20 v/v). An aliquot of the mixture was separated using Whatman filter paper and kept in refrigerator for carbohydrate analysis, while the other part was centrifuged at 1500 × g for 30 minutes. Pellets were obtained and discarded while the supernatant was collected for ammonium sulphate precipitation as described by Trowbridge (1974). The precipitated protein were pulled together and dissolved in 240ml of distilled water, the resulting mixture was concentrated by ultra-filtration (Millipore, India) at 1500g for 30 minutes before dialyses against 0.15M NaCl– 0.01M $NaPO_4$ buffer for 24 hours. The dialyzed sample was then collected in an empty bottle and stored at 4℃prior to assay for lectin activity.

2.6. Assay for Lectin Activity

Agglutination of red blood cells by the extract and the various fractions that were obtained during the purification steps was determined as described by Bing *et al*. (1967). A serial two-fold dilution of the lectin solution was mixed with 50µL of a 4% suspension of human erythrocytes in PBS (pH 7.2), at ambient temperature. The erythrocytes of human blood group A, B and O were fixed with 3% formaldehyde. The plate was left undisturbed for 60 minutes at ambient temperature in order to allow the agglutination of erythrocytes to take place. The heamagglutination titre of the lectin expressed as the reciprocal of the highest dilution exhibiting visible agglutination of erythrocytes was reckoned as one heam agglutination unit. Specific activity was expressed as the number of heamagglutination units per microgram protein (19).

2.7. Anti-Fungal Sensitivity Assay

The *in vitro* sensitivity of the isolated *Fusarium* species to the partially purified lectin was determined according to a modified disc diffusion method for antibacterial assay (20). Each *Fusarium* isolate was inoculated at the center of a 9cm Petri dish containing freshly prepared PDA and incubated at 22–24℃ for 3 days under a 12-hour light/darkness schedule. Four pieces of 3mm sterile paper discs were placed on the four cardinal points of the growing culture at distances of 0.5cm away from the boundary of the colony so as to give a square. Each of the four discs in a *Fusarium* culture was moistened with 5µL of the partially purified lectin suspension. This procedure was performed for the 10µL and 20µL treatments of paper discs in a *Fusarium* culture with the partially purified lectin. All cultures were treated in triplicates for each lectin treatment. Two sets of triplicate control plates were set up by impregnating the paper discs with 20µL of 0.15M NaCl in one set and 5µL of sterile distilled water in the other. The plates were then incubated as stated above for 4 additional days. The zones of inhibition were measured daily for 4 days.

2.8. Inhibition of Lectin-induced Heamagglutination by Various Carbohydrates

The ability of various carbohydrates to inhibit lectin-induced heamagglutination was investigated using a procedure that is analogous to the heamagglutination test described by Kuku *et al*. (2009). The sugars used were glucose, galactose, maltose, fructose, sucrose, lactose, raffinose, trehalose and sialic acid. Serial two-fold dilutions of each sugar sample were prepared in PBS. All the dilutions were mixed with an equal volume (50µL) of the lectin solution of known heamagglutination units. The mixture was allowed to stand for 60 minutes at ambient temperature and then mixed with 50µL of a 4% human erythrocyte suspension. The heamagglutination titres obtained were compared with a non-sugar containing blank. The minimum concentration of the sugar in the final reaction mixture which completely inhibited heamagglutination units of the lectin sample were obtained (19).

2.9. Effect of salts on Heamagglutinating Activity of Lectin

Nine salts [calcium chloride, $CaCl_2$; iron (III) sulphate, $Fe_2(SO_4)_3$; ferric chloride, $FeCl_3$; sodium sulphate, Na_2SO_4; copper sulphate, $CuSO_4$; magnesium chloride, $MgCl_2$; sodium chloride, NaCl; potassium chloride, KCl; potassium phosphate, KH_2PO_4] were evaluated for their inhibitory ability against heamagglutination induced by lectin. Serial two-fold dilutions of salt samples were prepared in PBS. All the dilutions were mixed with an equal volume (50µL) of the lectin solution of known heamagglutination units. The mixture was allowed to stand for 60 minutes at ambient temperature and then homogenized in 50µL of a 4% human erythrocyte suspension. The heamagglutination titres obtained were compared with a non-salt containing blank. The minimum concentration of the salt in the final reaction mixture which completely inhibited heamagglutination units of the lectin sample were obtained (19).

2.10. Effect of Temperature on Heamagglutinating Activity of Lectin

The method of Patrick *et al*. (2007) was adopted for testing the effect of various temperature regimes on the

agglutinating activity of lectin obtained from croton. The purified lectin was incubated in a water bath for 30 minutes at various temperatures: -10, -4, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100℃, and then cooled to 20℃. Heamagglutination assay was carried out as previously described.

2.11. Effect of pH on Heamagglutinating Activity of Lectin

The effect of different pH regimes (2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12) on the activity of lectin obtained from croton was determined by incubating the lectin in the buffers of different pH values. The pH values of 0.15M NaCl – 0.01M NaPO4 buffer were altered using concentrated HCl and 1M NaOH, and assaying for heamagglutinating activity. The control values were the agglutination titre of the lectin in PBS (pH 7.2).

3. Results

As a result of initial field observations carried out so as to determine the magnitude and severity of the infection by the *Fusarium* species, the cultivars of the garden croton have been described and categorized into THREE: namely, heavily susceptible (A), resistant (B) and tolerant (C) cultivars. In the category "A" the pathogen kills aborts the leaves and kills the twigs; whilst in the category "B" no signs of pathogenicity was observed. However, in the category "C" only necrotic leaf spots which do not spread or coalesce was the case. The leaves of *Codiaeum variegatum cv. royal* showed no growth of *Fusarium* while the diseased leaf discs of *C. variegatum cv. ovalifolium*, *C. variegatum cv. royal-like* and *C. variegatum cv. punctatum* showed growths of *Fusarium* species (Fig. 2). The isolates obtained from *C. variegatum cv. ovalifolium* were identified as *F. semitectum* and *F. lateritium* while those from *C. variegatum cv. royal-like* and *C. variegatum cv. punctatum* were all *F. lateritium*.

Lectin from *C. variegatum cv. royal* was found to agglutinate human erythrocytes and showed an activity that was non-selective to type of blood group (A, B, AB and O). The three concentrations of lectin exhibited antifungal activity towards all *Fusarium* isolates by inhibiting hyphal growth of the test isolates while the controls (NaCl and sterile distilled water) had no inhibitory effect on the isolates (Fig. 4). The data obtained from the studies for the inhibitory effects of different sugar concentrations on lectin activity of *C. variegatum cv. royal* lectin are presented in Table 1. It was observed that all concentrations of maltose, lactose and sucrose had no inhibitory or stimulatory effect on heamagglutinating activity of the lectin. The 100 and 200 mM/L glucose concentrations enhanced agglutination of human erythrocytes more than other concentrations of the sugar while the highest concentration of 800mM/L resulted in a complete loss of heamagglutination activity. In the presence of very low (25mM/L) and very high (400 and 800mM/L) galactose concentrations, we observed an increased binding of the lectin to human erythrocytes. On the other hand, fructose, trehalose and sialic acid did not inhibit or stimulate heamagglutination at very low concentration of 25mM/L, but showed a complete inhibition of the lectin's heamagglutination activity at higher concentrations of 100–800mM/L sugar. At concentrations of 50–200mM/L raffinose, heamagglutinating activity of the lectin was completely lost while an increase of the concentration of raffinose to 400mM/L enhanced the lectin's potential to bind to the red blood cells. Results similar to those observed in our study were reported by Perez (1995), who purified lectin from the seeds of *Erythrina costarisencis*.

The characterization and inhibition studies to define the salt specificities of lectin purified from *C. variegatum cv. royal* (Table 2) showed that all concentrations of $Fe_2(SO_4)_3$ and $FeCl_3$ did not inhibit or enhance the heamagglutinating potential of the lectin. On the contrary, the presence of 100–800mM/L $CuSO_4$ completely inhibited heamagglutination while the lower concentrations had no stimulatory effect either. The heamagglutinating potential of the lectin was at its peak in the presence of lower concentrations of $CaCl_2$ and NaCl, and as the concentrations reached 200mM/L for $CaCl_2$ and 400mM/L for NaCl the activity reduced until no significant influence of the salt on lectin-binding activity was observed at 400-800mM/L $CaCl_2$ and 800mM/L NaCl. Contrariwise is the higher stimulatory effect of increased concentrations of $MgCl_2$ (200-800mM/L) and KCl (400-800mM/L) towards the agglutination of human erythrocytes by lectins. Similar results were reported on lectin extracted from the seeds of *Cissus populnea* (16).

Figure 5 shows the heamagglutinating activity of lectin obtained from *C. variegatum cv. royal* under different temperature regimes. It was observed that there was no heamagglutination activity at incubation temperature of 20 ℃ and below. However, a rapid increase in heamagglutination and consequent peak level of activity was reached at incubation temperature of 30–40℃; this formed a plateau between the regimes. When the mix (lectin-erythrocytes) was incubated at higher temperatures (50 ℃ and above), a drastic reduction in activity was observed until the activity was completely lost at 80℃ and above. This therefore indicates that ambient temperature is best suitable for the binding activity of this *C. variegatum cv. royal* lectin. This finding is in contrast to that of the fructose-binding lectin purified from the gill of *Aristichthys nobilis* (24), where the lectin incubated at 50℃ for 30mins showed agglutination that was fourfold stronger than when it was incubated at ambient temperature.

Data on the effect of pH on lectin activity are presented in Fig. 6. Lectin activity was stable at two pH ranges of 5.0–6.0 (acidic) and 9.5–10.5 (alkaline). However, optimum activity was recorded at pH range of 5.0–6.0. This result may therefore suggest that the protein has two binding sites; one site more active at a slightly acidic pH range of 5.0–6.0 and the other at basic pH of 10.0. This finding is however, in contrast to that reported in which *Manila clam* lectin activity was stable between pH 6 and pH 9, and was

temperature-dependent (25). Our findings is also in contrast to other reports where *A. nobilis* lectin showed a decrease in agglutination activity on incubation at pH 6 for 30 mins therefore indicating its unstable nature under such conditions (24).

Table 1. Effects of sugar concentrations on the heamagglutinating activity of lectin from *Codiaeum variegatum cv. royal* lectin

Sugar	Concentration of sugars in mMol/L						Phosphate Buffered Saline
	25	50	100	200	400	800	
Maltose	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+
Glucose	+	+	+++	++	+	-	+
Galactose	+++	+	+	+	+++	+++	+
Fructose	+	-	-	-	-	-	+
Raffinose	+	-	-	-	++	+	+
Trehalose	+	+	-	-	-	-	+
Sialic acid	+	-	-	-	-	-	+

+ = agglutination (5–50 Hu/mg); ++ = agglutination (50–100 Hu/mg); +++ = agglutination (100 Hu/mg protein); - = No agglutination.

Table 2. Effects of salt concentrations on the heamagglutinating activity of *Codiaeum variegatum cv. royal* lectin

Salt[a]	Concentration of salts in mMol/L						Phosphate Buffered Saline
	25	50	100	200	400	800	
$CaCl_2$	+++	+++	+++	++	+	+	+
$Fe_2(SO_4)_3$	+	+	+	+	+	+	+
$FeCl_3$	+	+	+	+	+	+	+
Na_2SO_4	+	+	+++	++	+	-	+
$CuSO_4$	+	+	-	-	-	-	+
$MgCl_2$	+	+	+	+++	+++	+++	+
NaCl	+++	+++	+++	+++	++	+	+
KCl	+	+	+	+	++	+++	+
KH_2PO_4	++	+	+	-	-	-	+

+ = agglutination (5–50 Hu/mg); ++ = agglutination (50–100 Hu/mg); +++ = agglutination (100 Hu/mg protein); - = No agglutination.
[a]Salt: $CaCl_2$, calcium chloride; $Fe_2(SO_4)_3$, iron (III) sulphate; $FeCl_3$, ferric chloride; Na_2SO_4, sodium sulphate; $CuSO_4$, copper sulphate; $MgCl_2$, magnesium chloride; NaCl, sodium chloride; KCl, potassium chloride; KH_2PO_4, potassium phosphate

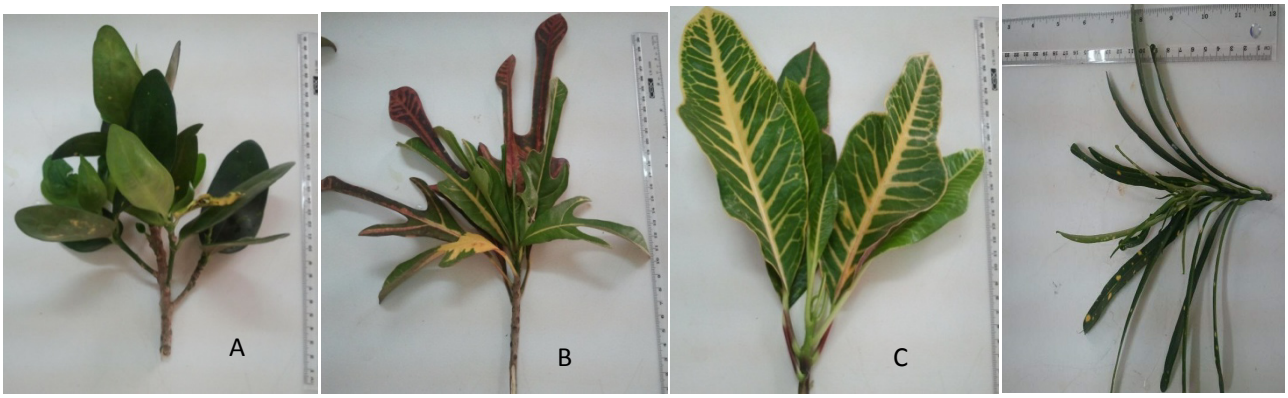

Figure 1. (A-D): Cultivars of Codiaeum variegatum used in this study. A) C. variegatum cv. royal B) C. variegatum cv. royal-like C) C. variegatum cv. punctatum D) C. variegatum cv. ovalifolium

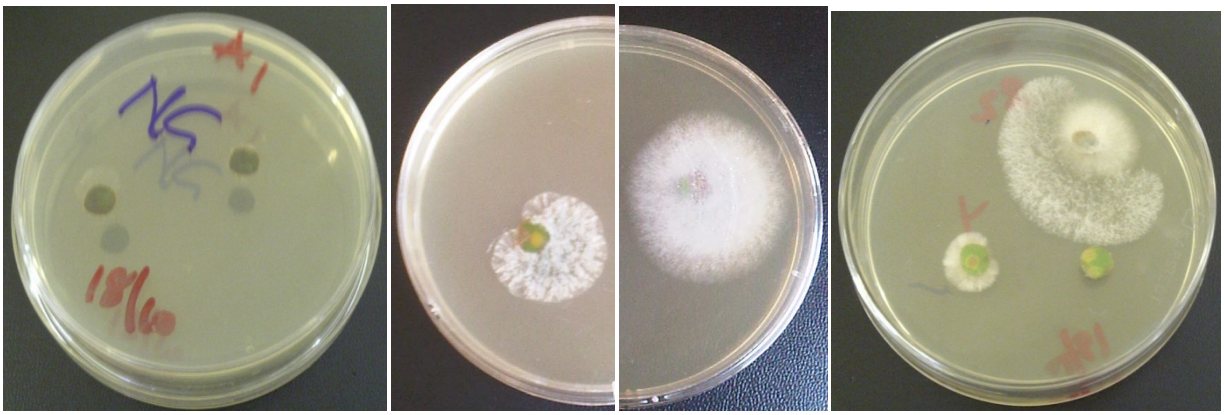

Figure 2. (A-D): Leaf discs of *Codiaeum variegatum* cultivars showing growths of pathogenic *Fusarium*. A) *C. variegatum cv. royal* did not show growth of *Fusarium*. B & C) *C. variegatum cv. royal-like* and *C. variegatum cv. punctatum* both show growths of *Fusarium lateritium* D) *C. variegatum cv. ovalifolium* shows growth of *F. semitectum*

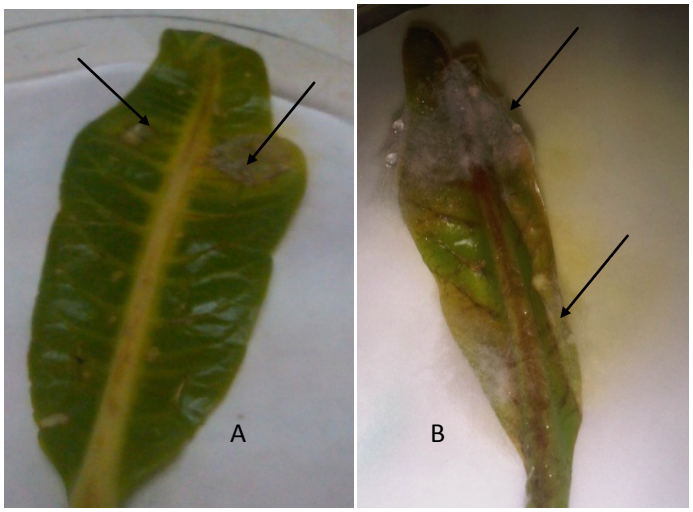

Figure 3. (A-B): Leaves of *Codiaeum variegatum* cultivars showing disease symptoms caused by *Fusarium* species. A) localized symptoms of mild discoloration and necrosis in *C. variegatum cv. royal-like* induced by sub-tissue growth of *Fusarium lateritium*. B) severe non localized chlorosis and necrosis in *C. variegatum cv. ovalifolium* induced by actively growing mass of *F. semitectum*

Figure 4. (A-C): Inhibitory activity of lectin on *Fusarium semitectum* isolated from *C. variegatum* cultivars. A) Control plate: 0.15M buffered saline (NaCl) impregnated discs showed no inhibitory effect. B) Test plate: 10µL lectin solution impregnated discs showed antifungal activity. C) Test plate: 20µL lectin solution impregnated discs showed antifungal activity

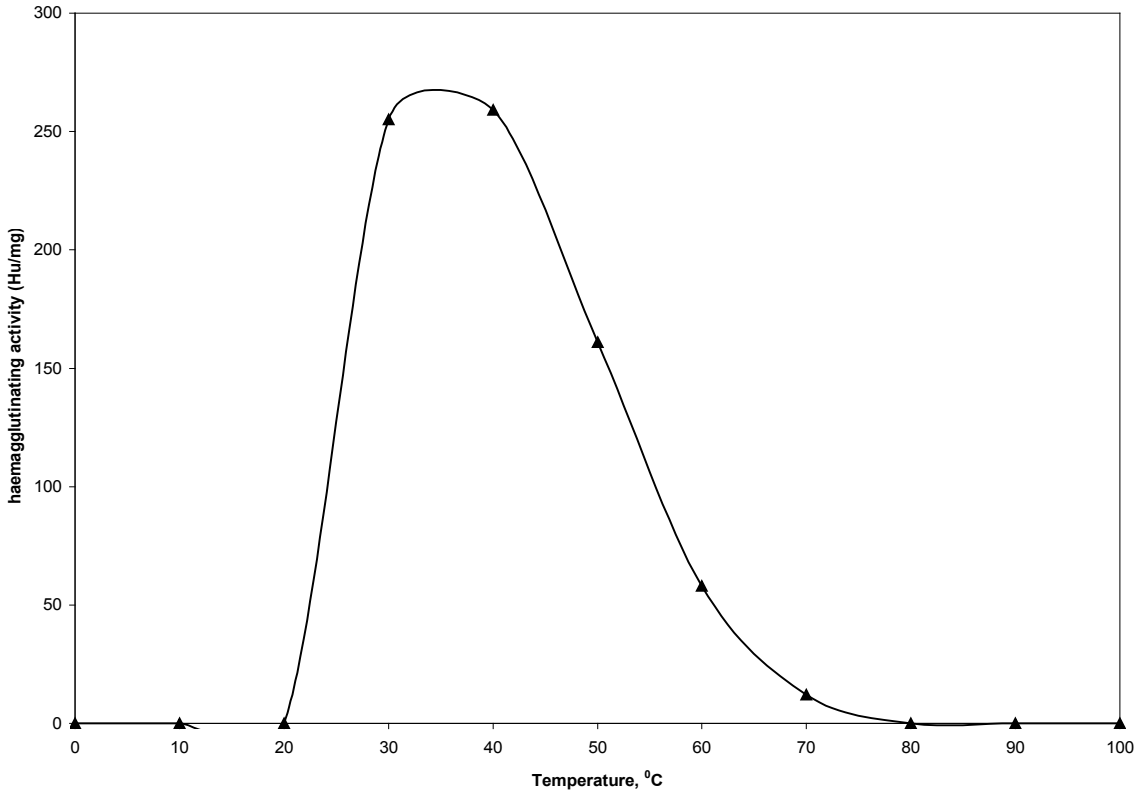

Figure 5. Effect of temperature on the heamagglutinating activity of *Codiaeum variegatum cv. royal* lectin

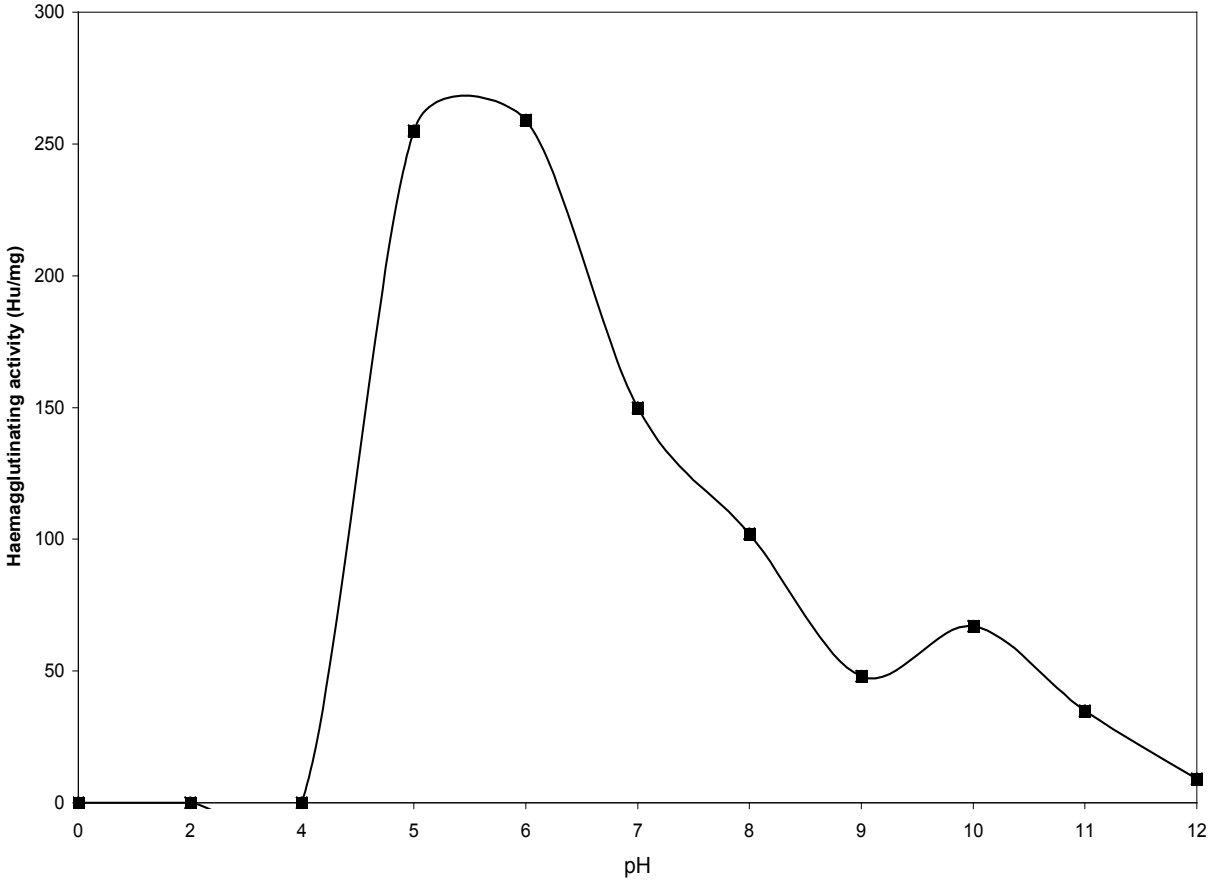

Figure 6. Effect of pH on the heamagglutinating activity of *Codiaeum variegatum cv. royal* lectin

4. Discussion

In this study, the inhibitory activity of the lectin was not concentration dependent because there was no significant difference in the zones of inhibition caused by the lectin concentrations against the isolates although the zones produced by the 20µL lectin concentration were higher than those of the lower concentrations (data not shown). This finding supports the report of Awoyinka & Dada (2011) and the wider inhibition zone may be due to an increased concentration translating into a higher antifungal activity. It has been suggested that plant lectins may have important roles according to their abundance, including in the immune defense, and also that lectins have been co-opted for several functions during evolution (24). The role of lectin in defense mechanism of plants may have evolved from the ability of the lectins to agglutinate and immobilize microorganisms. The supporting evidence for this proposed role in defense against pathogens falls into two categories: the presence of lectins at potential sites of invasion by infectious agents and the binding of lectins to various fungi and their ability to inhibit fungal growth and germination (26). The ability of *C. variegatum* lectin to inhibit fungal growth suggests that it may play an important role in immobilizing invading micro organism particularly fungal infections. The inhibition of fungal growth may have occurred through lectin binding to hyphae resulting in poor absorption of nutrients as well as by interference on spore germination process. This by interpretation means that resistance occurs as soon as the pathogen arrives on the leaves, a phenomenon referred to as a Systemic Acquired Resistance (SAR) due to the production of phytoallexins (27). When plants respond to attacks by either other plants especially lower plants called cryptogams, as pathogens or animals (herbivores) plants counter these threats with inherent defence systems that deter herbivory and or prevent infections or combat pathogens that infect. Defense systems could be physical, genetic, chemical or biochemical, that is, by ability of the plant to recognize invading pathogen; especially if unsuccessful. Occasionally, a kind of compromise might have been evolved between the plants and pathogens. Under such cases, the pathogen simply ensures its own survival without severely damaging or killing the plant wholly or partially (27). The relationship between plant lectins and phyloallexins though not studied in the investigation it might be another classical example of the fact that in plants, there are usually more alternative biochemical pathways evolved to regulate or control processes that ensure survival when plants are under stress, unlike what obtains in animals.

The isolation of *Fusarium* species from the diseased leaves of susceptible cultivars of *C. variegatum* investigated in this study (Fig. 3) is in line with a previous report (11). This study has shown that *Fusarium* species are major phytopathogens of the garden croton plant and their spectrum of pathogenicity is dependent on the presence or absence of lectins. The ability of lectin from disease resistant *C. variegatum* to inhibit the *Fusarium* species suggests that it may play an important role in immobilizing invading microorganisms particularly fungal infection. In addition, the carbohydrate-binding site of *C. variegatum* lectin may be vital in this activity, being responsible for the recognition of the fungi. The resistant cultivar of *C. variegatum* used in this study may be a suitable candidate for the prevention and treatment of fungal infections.

5. Conclusions

This study has shown that Fusarium species are major phytopathogens of the garden croton plant and their spectrum of pathogenicity is dependent on their Fructose binding specific lectins and their clinical importance. In addition, the resistant cultivar of C. variegatum (cv. royal) used in this study may be a suitable candidate for the prevention and treatment of these fungal infections, through graftage and or hybridization. Although this study is not a comprehensive exercise on plant defenses against pathogens, the "lectins concept" is probably just one of these defenses.

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