بسم الله الرحمن الرحيم

Sudan University of Science and Technology

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*IN-VITRO*ANTIFUNGAL ACTIVITY OF GINGER *ZINGIBEROFFICINALE* ETHANOLIC EXTRACTSAGAINST PLANT PATHOGENIC FUNGUS"*FUSARIUMOXYSPORIUM"*

تأثير المستخلص الإيثانولي النباتي لجذور الزنجبيل على نمو الفطر *FUSARIUM OXYSPORIUM*

A thesis submitted in partial fulfillment of the requirements for B.Sc. Honors in plant protection

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الآية

قال تعالي:

(وَظَلَّلْنَا عَلَيْكُمُ الْغَمَامَ وَأَنْزَلْنَا عَلَيْكُمُ الْمَنَّ وَالسَّلْوَى كُلُوا مِنْ طَيِّبَاتِ مَا رَزَقْنَاكُمْ وَمَا ظَلَمُونَا وَلَكِنْ كَانُوا أَنْفُسَهُمْ يَظْلِمُونَ (٥٧))

صدق الله العظيم

سورة البقرة (57)

Dedication

To my parents

To my friends and to everyone who helped me in this research

With Love

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I would like very much to render His Almighty Allah who gives me the power and health to complete this work.

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الملخص العربي

اجريتهذهالدراسهتحتظروفالمختبربقسموقايةالنبات ,(معملامراضالنبات) كليةالدراساتالزراعية , جامعهالسودانللعلوموالتكنولوجيا (شمبات) لدراسهتاثيرالمستخلصالكحوليلجذور الزنجبيلوالمبيدالفطريتوباس100 ملعلىنموفطر*F. oxysporium* المسببلمرض العفن الجاف على درنات البطاطس.

استخدمتثلاثهتراكيزمنالمستخلصلرايزومالزنجبيل (50%- 25% - 12.5%)وكذلكاستخدمالمبيدالفطريتوباس100 مل أضافةالىالشاهد. تمتقييمالاثرالتثبيطلهذهالتراكيزبتسجيلنسبهتثبيطنموالفطر.

اوضحتالنتائجانكلتراكيزالمستخلصالكحوليالنباتالمختبرةوالمبيدالفطرىقداظهرتتاثيرمعنوىضدالفطر محل الدراسةمقارنهبالشاهد. تراكيزالمستخلصالكحوليوالمبيدالفطرىقدتفاعلتكلعلىحدة ضدالفطرالمختبرمعتاثيرواضحللمستخلصاتمنالمبيدالفطرى .

أوضحت النتائج أن تركيز المستخلص الكحولي (50%) كان هو التراكيزالاعليمعنويا (P=0.05) لتثبيط نمو الفطر بنسبة وصلت 100% مقارنة بالمبيد الفطري والشاهد. وقد أوضحت الدراسة أيضاً أن التراكيز (12.5,25%) قد أعطت نتائج اقل تثبيطا لنمو الفطر محل الدراسة بفرق معنوي على مستوى (P=0.05) حيث سجلت نسبة التثبيط في التركيزين 88.6% و 78.7% على التوالي مقارنة بالمبيد الفطري والشاهد.

وتخلصهذهالدراسةالىانالزنجبيليحتويعليبعضالمكوناتالتيلهاالمقدرةعليتثبيطالفطرالامرالذييمكناعتبا رهذوفائدةلاستمرارهذاالنوعمنالبحوثلتحديدالموادالطبيعيةالتيتتضمنهاالزنجبيل وتاثيره على الفطر خارج العائل. وعليه نوصى باستمرار البحث لدراسة تأثير الزنجبيل على الفطر داخل العائل.

ABSTRACT

The present investigation was undertaken under laboratory of Plant protection Department, College of Agricultural Studies, Sudan University of Science and Technology, to study the effect of ethanol extract of ginger and fungicide topas (100 ml) on the growth, of the fungus, *Fusariumoxysporium*that causing dry rot onpotato tubers.

Three concentrations of ethanol extract of ginger rhizome (50,25and12.5%), and standard fungicide (Topas100 ml) were used in addition to the untreated control. The assessment of their inhibitory effect against the pathogen was recorded through the fungal growth inhibition.

The results showed that the concentrationof the ethanol extract (50%) of ginger showed the higher significant inhibitory (100%) effect on the fungus growth compared to the standard fungicide and the control (p=0.05). However, the ginger ethanol extracts concentration 25% and 12.5% reacted differently showinglowerinhibitory effect on the fungus growth aiming at 88.6% and 78.8% respectively, with a significant difference at 0.05 level compared to the untreated control.

Generally, the results showed that the antifungal activity increases with increase in extract concentration. Accordingly we recommend continuing the research to confirm our findings to control the fungus *in vivo*.

CHAPTER ONE

INTRODUCTION

Potato plant (*Solanum tuberosum* L.) is a member of the family Solanaceae that includes also eggplant, tobacco and tomato. Potato is an important crop worldwide and ranks fourth in production among food crops after maize (*Zeamays* L.), rice (*Oryza sativa* L.), and wheat (*Triticum aesitvum* L.) (FAOSTAT data, 2006). Its importance is increasing due to the rising world population, the capability of potatoes to grow well in adverse conditions, and its high nutritional value with an annual production of 3.6 x108tones (Hamilton, 2005).The crop was originally domesticated independently in multiple locations.

In Sudan, the crop is cultivated in wide area around large Cities along the Nile and on seasonally flooded plains (FAO, 1999). However, the area around Khartoum accounts for over 70% of the country's potato production (Ibrahim, 1988). On the other hand, potato is also cultivatedin Jebel Marra in the far west and it is locally known as Zalinge potato.

The losses caused by diseases and insects constitute the major constraints that facing the production of potato worldwide and among these, the most wide spread and important are fungi, affecting tubers and vegetative parts. One of the main fungal pathogens that attack potato is Fusarium dry rot, which is a worldwide economic problem. There are many species of *Fusarium* reported to cause dry rot of potato Worldwide (Nielson, 1981) of which *Fusarium solani* has been reported as the most pathogenic *Fusarium* species causing potato dry rot.

The disease affects tubers in storage and seed potato pieces after planting. Hanson *et al*, (1996) reported that *Fusarium* dry rot of feed tubers could cause crop losses up to 25%, while more than 60% of tuber can be infected in storage.

The miss use of chemical pesticides to control various pests and pathogenic microorganisms of crops plants is causing health hazards through their residual toxicity, much attention is being focused on the alternative methods of pest control (Ali, 1996).

Natural plant extracts have been recommended as suitable alternative choices to synthetic chemicals to control diseases and pests of crops.

Based on the foregoing and considering the adverse and alarming effects of synthetic pesticides on environment and natural habitats, this study was undertaken to find out alternative and nontoxic biological control agents to control the *Fusarium*the causal agent of the dry rot of potatoes*in vitro*. Aiming at investigating the antifungal activity of some higher plant extracts and fungicide against the said fungus under laboratory conditions

withfollowing objectives:-

- To explore the antifungal potentials of some higher plants crude extract against *Fusarium* dry rot of potato
- To evaluate the efficacy of systemic fungicide on fungal growth

CHAPTER TWO

LITERATURE REVIEW

2.1 Fusarium *Oxysporum*

*Fusarium*species causes a huge range of diseases on an extraordinary range of host plants. The fungus is a soil borne pathogen, airborne or carried in plant residues and can be recovered from any part of the plant from the deepest root to the highest flower (Booth, 1971; Summeral*et al.*2003 .(Fusarium wilt caused by *Fusarium oxysporum* disease that causes serious economic loss (Agrios, 2005). The fungus causes vascular wilts by infecting plants through the roots and growing internally through (Bowers and Locke, 2002).

2.1.1 Classification

Kingdom: Fungi

Division: Ascomycota

Class: Sordariomycetes

Order: Hypocreales

Family: Nectriaceae

Genus: Fusarium

Species: Fusarium oxysporum

Fusarium oxysporum strains are ubiquitous soil inhabitants that have the ability to exist as saprophytes and degrade lignin (Rodriguez *et al*., 1996, Sutherland,*et al*., 1983) and complex carbohydrates (Christakopoulos *et al*., 1995/1996), associated with soil debris. Although the predominant role of this fungus in native soils may be as harmless or even beneficial plant endophytes or soil saprophytes, many strains within the *F. oxysporum* complex are pathogenic to plants, especially in agricultural area.

This remarkably diverse and adaptable fungus has been found in soils ranging from the Sonorant Desert, to tropical and temperate forests, grasslands and soils of the tundra. (Stoner, 1981).

2.1.2 Description

Fusarium oxysporum is a common soil inhabitant. Booth (1977) stated that *F. Oxysporum* has a colorless mycelium at first but with age, it becomes creamy in colors pale yellow, pale pink or purplish. These colors give the characteristic culture pigment within the plant.

The fungus produces three types of asexual spores, microconidia, macroconidia and Chlamydospores. The macroconidia are straight to slightly curved, slender thin walled usually with three or four septa, of a foot shaped cell. They are generally produced on conidiophores by division. They are important in secondary infection. The micro conidia are ellipsoidal and either have no septum or single one. They are formed from phial ides in false heads by secondary infection (Agrios, 2005).

The chlamydispores are globes and have thick walls. It is formed from hyphae or alternatively by the modification of micro cells. Conidia considered as endurance organs in soil where they act as inoculums in primary infection.

The teleomorph or sexual reproductive stage of *F. oxysporum* is unknown untilrecently, Agrios (2005) reported that the teleomorph of the fungus was found and known as *Gibberella sp*.

2.1.3 Distribution

Worldwide, pathogenic races may have different distribution, defined by range - common in temperature regions; North and South America, Europe, Africa, Australia and New Zealand .those are Fusarium in *linumspp.* and *Gossypiumspp.* as reported.

Whose strains represent some of the most abundant and widespread microbes of the global soil microflora, (Gordon, and Martin, 1997).these remarkably diverse and adaptable fungi have been found in soils ranging from the Sonorant Desert, to tropical and temperate forest, grassland and soils of the tundra. (Stoner, 1981).*F.oxysporium* strains are ubiquitous soil inhabitants that have the ability to exist as saprophytes, and degrade lignin. (Rodriguez *et al* 1996) and complex carbohydrates (Christakopoulos, *et al*, 1996) Associated with soil debris.

The wilt disease was found to be more serious in low rainfall areas, were the weather condition are favorable for disease development (Khan, 1980).

2.1.4 Host Range

The most important Fusarium wilt pathogens have wide range of host and including numerous forma specials, some of them contain two or several pathogenic races, causing devastating wilt diseases and many are seed borne as listed by Andersen (1974) for the following hosts *Alliumscannabis*. *Betavulgaris*, *Cucumissativa*, *Phaseolusvulgaris* and *Pisumsativum*.

F.oxysporum is one of the major causal agents of wilt disease (Nene *etal*; 1991). The disease is prevalent in most Tomato growing countries and is a major disease. It is seed and soil borne disease .The fungal pathogen *F.oxysporium* affects a wide variety host of different age Tomato, Tobacco, Legumes, Cucurbits, Sweet Potatoes, Chickpea and Banana are a few of the most susceptible plant, but it also affects other herbaceous plants (Pan Germany, 2010).

2.1.6 Control

2.1.6.1 Cultural control:

The culture control is the only practical measure for controlling the diseases in the field. The wilt fungus is so widespread and so persistent in soils that seedbed sterilization and crop rotation although always sound practices but are of limited value. Soil sterilization is too expensive for application but it should be always practiced for greenhouse grown tomato plant (Agrios 2005).

Moreover, use of healthy seed and transplants is of course mandatory, and hot water treatment of seed suspected of being infected should precede planting (Agrios, 2005).

As mentioned above, Fusarium wilts affect and cause severe losses on most vegetable and flowers, several field crops such as cotton, Tobacco, banana, plantain, coffee, sugarcane and a few shade trees. Fusarium wilts are most severe under warm soil conditions and green house (Agrios, 2005). Most Fusarium wilts have diseases cycles and develop similar to those of the Fusarium wilt of tomato.

2.1.6.2 Botanical controls

The antifungal effect of certain medicinal and aromatic plants extracts have been investigated by many workers (Singh and Dived, 1987; Henrique and Singh 1990). Thus, the development of new and different antimicrobial agents more safe has been a very important step (Agrafotis, 2002). However, a number of researchers studied the step of validation of traditional uses of antimicrobial compounds in higher plants. Accordingly, the effect of different plants extracts on the germination and growth of many fungal pathogens have been reported (Agrafotis, 2002).

The use of plant extracts for controlling Fusarium wilt, cultural practices and the use of other methods are the most common strategies. However, they are either not available or effective. The uses of natural products for the control of fungal diseases in plant are considered as an interesting alternative to synthetic fungicides due to their less negative impacts on the environment. Plant extracts or plant essential oils have been tested against *F.oxysporum* species for inhibitor effect and control efficacy under greenhouse condition (Bowers, and Locke, 2000). If natural plant products can reduce populations of soil borne pathogens and control diseases development, than these plant extracts have potential as environmentally safe alternatives and as component in integrated pest management programs. Chand and Singh (2005), reported that the plant extracts, of*Calotropisprocera, Eucalyptusglobulins*, *Jatrophamultifida*, *Azadirachtaindicia*, *Alliumsativum* were significantly pronounced in reducing wilt incidence in *Cicerarietinum* L. Mycelial growth of various Fusarium species were inhibited by the plant extracts of, *Azadirachtaindica* , *Cinnamomumcamphora*, and *Ocimumsanctum* (Prasad and Ojha,1986); *Agaveamericana, Cassianadosa* (Reddy and Reddy,1987); *Azadirachtaindica*, *Atrophabelladonna*, *Calotropisprocera, Eucalyptusamgdalline, Ailanthusexclsa* and *Lantanacamara* (Banal and Rajesh, 2000; Nwachukku and Umechuruba, 2001).

2.1.6.3 Chemical control

Presently, Anon (1994) reported that methyl bromide fumigation is used extensively for tomato production in some geographical areas in addition to reducing or eliminating soil borne diseases like Fusarium wilt . Fumigation allows more rapid transplant growth allowing for earlier harvesting and optimizes fresh markets. The use of methyl bromide may be curtailed in near future and alternative chemicals are being examined.

2.2Ginger (*Zingiber officinale):*

2.2.2 Classification

Kingdom : Planate

Subkingdom : Tracheobionta

Super division : Spermatophyte

Division : Magnoliophyta

Class : Liliopsida

Subclass: Zingiberidae

Order : Zingiberales

Family : Zingiberaceae

Genus ::*Zingiber*

Species : *Zingiberofficinal*

Ginger plants have known to originate in South East Asia, probably in India (Burkill, 1990). One of the species under this group is. *Zingiber officinale* that is known to possess markedly highantioxidant potential compared to other species studied till date (Nan-Chen *et al*., 2008), is cultivated in several countries such as in Australia, Bangladesh, Haiti,Jamaica, Japan, Nigeria, Sri-Lanka, and South East Asian countries including China, Nepal, Malaysia, NorthKorea, Indonesia and India (Wu and Larsen, 2000). In addition toavailability under cultivation, large populations of these plants are also available as land races in the wild, withEastern and North-Eastern India. Rhizomes of the plants are used as spice whereas both rhizomes and leaves provide important sourceof medicine. Several landraces of *Zingiber officinale* have been identified by local communities to be elite withrespect to medicinal and spice value (Sanjeev *et al*.,2011).Some of genotypes of *Zingiber officinale* areparticularly valued for their non-fibrous rhizomes that are likely to provide high content of bio-molecules in thehigher content of soft tissue (Kizhakkajii and Sasikumar, 2011). Being vegetative propagated by rhizomes thatconstitute the plant part for spices and medicines, such plants run the risk of over exploitation in the wild, thisadds to the urgent need for Documentation Evaluation and Conservation of these plants. Understanding genomeprofiling vis-à-vis antioxidant (medicinal) potential of wild population of ginger plants for

screening hithertounexplored medicinal plants that would help to bring underutilized germplasm to cultivation focus, would repaycareful investigation.

2.2.1 Medicinal uses

According to the American Cancer Society, ginger has been promoted as a cancer treatment "to keep tumors from developing," but "available scientific evidence does not support this." They add: "Recent preliminary results in animals show some effect in slowing or preventing tumor growth. While these results are not well understood, they deserve further study. Still, it is too early in the research process to say whether ginger will have the same effect in humans.

CHAPTER THREE

MATERIAL AND METHODS

This study was conducted in the laboratory of plant pathology, Department of Plant Protection, College of Agricultural Studies (CAS), Sudan University of Science and Technology (SUST) during April 2016. The study was conducted to isolate and control the fungus Fusarium *oxysporum.* by using ethanolic extract of the ginger (*Zingiber officinalis*) rhizomes collected from the market, and to explore the methods of control under laboratory conditions where temperature around 25^0C.

3.1 Materials, tools and equipment used in the study

Gloves – camera - marker pen - Petri-dishes - sensitive balance – incubator – needle – flame - sodium hypochlorite - - Ethanol 95% - Para film - Site of equipment - laminar flow - Light microscope - Slide and slide covers - aluminum foil - water bath - potato dextrose agar (PDA) - filter papers - medical cotton.

All materials except the ginger rhizome, which used in this experiment, were sterilized using 1% sodium hypochlorite and dried under the shade and weighted about 100grams in soxlate after mixed by ethanol 95%, sterilized Petri dishglass were used.

3.3.4 Preparation of ethanol extracts of ginger

Thee ethanolic extractof ginger powder were prepared by adding 100 grams of the rhizome powder to 90 ml ethanol in a conical flask 250ml. The mixtures were shaken every 8 hours until 24 hours at room temperature. The mixture was then strained using a light cloth, and then filtered through filter paper what man No. 1 and stored until the experiment time**.**

3.3.5 Test procedure

The PDA media was amended with the required concentration (12.5, 25 and 50%) before being solidified in a conical flask of 100 ml, agitated before pouring it into sterilized Petri dishes. Three petri dishes glasses were assigned for each concentration and left to solidify. The other three plates with PDA medium mixed by extract concentration were served as control.

The Petri dishes of each concentration were inoculated using sterilized filter paper disc dipped in a fresh culture suspension of corresponding fungus and placed at the center of the plate. In case of the control the disc was treated with sterilized distilled water and placed at the center of Petri-dishes. Inoculated Petri dishes were then incubated at 25 C^0 for 4 days. The growth of the fungus was recorded every day. Treated plates were arranged in a randomized complete block design.

The effect of each extracts was evaluated as percentage of reduction in diameter of fungal radial growth (R) where:-

$$R = \frac{dc - dt}{Dc} \times 100$$

- Where
- R = Percentage reduction of the growth,
- dc= diameter of controlled growth and
- dt= diameter of treated growth.

3.4. Statistical analysis

The data obtained was statistically analyzed according to analysis of variance (one way-ANOVA), Duncan's Multiple Range Test, L.S.D test was used for means comparison.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Effect of Zingiber ethanol extractsand fungicide topas (100) on the radial growth of*F.oxysporium:*

The results (Table 1) showed that theethanolic extracts of all plants tested in addition to standard fungicide Topas100 ml had effects on the fungal growth after three days from inoculation. Furthermore, the percentages fungal growth inhibition was significantly high compared to the control.

The Zingiber was more effective of the growth dome of*F.oxysporium.* Moreover, the highest concentration of the plant extracts (5o %) and topas100 ml gave significantly higher inhibition zones percent (100%, 100%, 100%). Generally, the results showed that the botanical antifungal activity increase with increasing of extract concentration.

Generally,all the plant extracts exhibited different degrees of antifungal activity against *F.oxysporium*. Thegrowth of *F.oxysporium* was inhibited by 25% and 12.5% tested concentrations of ethanolic extracts compared with control, the correspond inhibition ranging from 88.6% - 78.7% respectively.

These results are in accord with the results obtained by Ababutain (2011). Our results revealed that ginger ethanol extract of different concentrations used in the study are all promising in controlling the fungus *Fusariumoxysporumin vitro.*

We recommend the continuity of this research to elaborate on the fungus inhibition *in vivo*.

Table,(1) :- Effects of ethanol extracts of Ginger and Topas (100ml) fungicide on the linear growth (inhibition zone %) against *F. oxysporium*of 4 days after inoculation .

Treatment concentrations. (%)		Inhibition zone means (%)			
		Day1	Day2	Day3	Day4
Ginger	50	9.467A	9.467A	8.500B	7.733AB
	25	7.967B	7.667B	7.267AB	6.300BC
	12.5	6.433C	6.433B	4.800B	5.133C
Topas 100ml		10.0A	10.0C	10.0A	10.0A
Control		0.7D	0.7D	0.7C	0.7D

Any two mean value (s) bearing different superscripts (s) are differing significantly (p<0-0.5).

- **Data transformed using square root transformation($\sqrt{X + 0.5}$) before analysis.**

CHAPTER FIVE

REFERENCES

Ababutain, I.M. (2011). Antimicrobial Activity of Ethanolic Extracts From Some Medicinal Plant. Australian Journal of Basic and Applied Sciences, 5(11): 678-683.

Agrafiotis D.K.; Bone, R. and Salemme, F.R (2002), soil R .method of generating chemical compounds having desired properties .US patent 6:434,490 August 13.

Agrios, G.N. (2005) Environmental effect is on development of the infectious disease. (In)plant pathology.5th end, Elsevier Acad .press Burlington, mass, USA pp251-262.

Ali, M.E.K. (1996) .A review of wilt and root –rot diseases of food legumes. In production and important of cool-season food legumes.In the Sudan proceedings of the National Research Review workshop, (S.H Salih, O.A.A.Ageeb, M.C.Saxena, M.B.Soih, Ed), Agricultural Research Corporation, Sudan /international Center for Agricultural Research in the Dry Areas, Syria/ Directorate General for international Cooperation, the Netherlands, 153-168.

Anon (1994).UNEP .methyl Bromide Technical options committee. Montreal protocol on substances that deplete .the ozone Layer: 1994 report of the MBTOC Environment protection Agency 430/K94 /029

Bansal, K.R. and Rajesh, K.G, (2000) Evaluation of plant extracts against *Fusariumoxysporium*, wilt pathogen of fenugreek, Indian .J. Phytopathol. 53:107_108.

Booth C. (1971).The genus Fusarium.Commonwealth Mycological Institute, Kew.

Bowers and J.C.Locke (2002). Effect of botanical extracts on the population density of *Fusariumoxysporium* in soil control of Fusarium wilt in the greenhouse, plant Disease, 84:300_305

Burkill, I. H. (1990). A Dictionary of the Economic Products of the Malay Peninsula,Kuala Lumpur, Ministry of Agriculture and Co-operatives.

Chand, H. and Singh .S. (2005).control of Chickpea wit (*FusariumoxysporiumF.spCiceri*) using bio agents and plant extracts. Indian J.Agric Sci. 75:115_116.

Christakopoulos, P., Kakos, D., Macris, B.J., Claeyssens, M. and Bhatt, M.K. 1995.Purification and mode of action of a low molecular mass endo-1, 4-B-D-glucanase from *Fusariumoxysporium.* J. Biotechnol. 39:85-93.

Christakopoulos, P., Nerinckx, W., Kakos, D., Macris, B. and Claeyssens, M. 1996. Purification and characterization of two low molecular mass alkaline xylanases from *Fusariumoxysporium* F3. J. Biotechnol. 51:181-180.

FAO, (1999). Food and Agriculture Organization of theUnited Nations.

FAOSTAT, (2006). Food and Agriculture Organization of theUnited Nations.

Gordon, T.R. and Martin, R.D. 1997. The evolutionary biology of *Fusariumoxysporum*.Annu. Rev. Phytopathol. 35:111-128.

Henrique, A.K.and Singh, H.B. (1990). Antifungal action of Lemongrass oil on some soil borne plant pathogens. Indian performer. 34(3):232_234.

Hanson, L.E, Schwager, S.J and Loria, R (1996) Sensitivity to thiabendazole in Fusarium Species associated with dry of potato psychopathology, 86: 378- 384.

Ibrahim, A.H.I. (1988). Incidence and elimination of potato viruses in the local stock "Zalinge". A Thesis, Khartoum University.

Khan, I. U. (1980). Chickpea pathology in Pakistan .in: proceeding of the international workhop on chickpea improvement, ICRISAT, Hyderabad, India, pp_257.

Kizhakkayil, J., & Sasikumar, B. (2011). Diversity, characterization and utilization of ginger: a review. PlantGenetic Resources, 9, 464-477. http://dx.doi.org/10.1017/S1479262111000670

Nan Chen,I., Chen-Chin, C., Chang-Chai, N., Chung-Yi, W., Yuan-Tay, S., &Tsu-Liang, Chang. (2008).Antioxidant and Antimicrobial Activity of Zingiberaceae Plants in Taiwan. Plant Foods Hum Nutr, 63, 15-20.

Nelson, P.E., Dignani, M.C. and Anaissie, E.J. 1994. Taxonomy, biology, and clinical aspects of Fusariumspecies.Clin.Microbiol.Rev. 7:479-504.

Nene, Y.L., Reddg, M.V.; Haware, M.P.; Ghanekar, A.M and Amin, K. S. (1991).Field diagnosis of Chickpea diseases and their control .in: information Bulletin no 28. Ed by .crops Res inst _for the semi A rid Tropics, patancheru, India.

Nielson, L.W. (1981) Fusarium dry rots: In compendium of potato Diseases, 58-60. 17.

Nwachukku, E.O.andUmechurba, C.I. (2001). Antifungal activities of some leaf extracts on seed germination and seedling emergence .J.APP.SCI.Envirom. Manage. 5:29_32.

Pan Germany (2010) Biocides .risks and alternatives. Hamburg Hyperlink: http://WWW.pangermany .org /download /biocides S risks and alternative _PDF

Parsed, H.K. and Ojha, N.L. (1986). Antifungal evaluation of leaf extracts for the control at some cucurbitaceous fruit rot diseases. Indian Phytopathol .39:135.

Reddy, V .K. and Reddy, S.M. (1987).screening of indigenous plants for their antifungal principle. Pesticides, 2:17_18.

Rodriguez, A., Perestelo, F., Carnicero, A., Regalado, V., Perez, R., De la Fuentes, G. and Falcon, M.A.1996.Degradation of natural lignin's and lignocelluloses substrates by soil-inhabiting fungi imperfecti.FEMS Microbial. Ecol. 21:213-219.

Sanjeev, S., Roy, A. R., Iangrai, B., Pattanayak, A., &Deka, B. C. (2011). Genetic diversity analysis in thetraditional and improved ginger (ZingiberofficinaleRosc.) clones cultivated in North-East India. ScientiaHorticulturae, 128(3), 3182-188.

Singh, R.K. and Dived, R.S. (1987).effect of oils on *Sccerotiumrolfsii* causing root rot of barley .Indian phytophol. 40:531_533.

Stoner, M.F. 1981.Ecology of Fusarium in no cultivated soils. Pages 2076-286 in: Fusarium: Diseases, Biology, and Taxonomy. P.E.

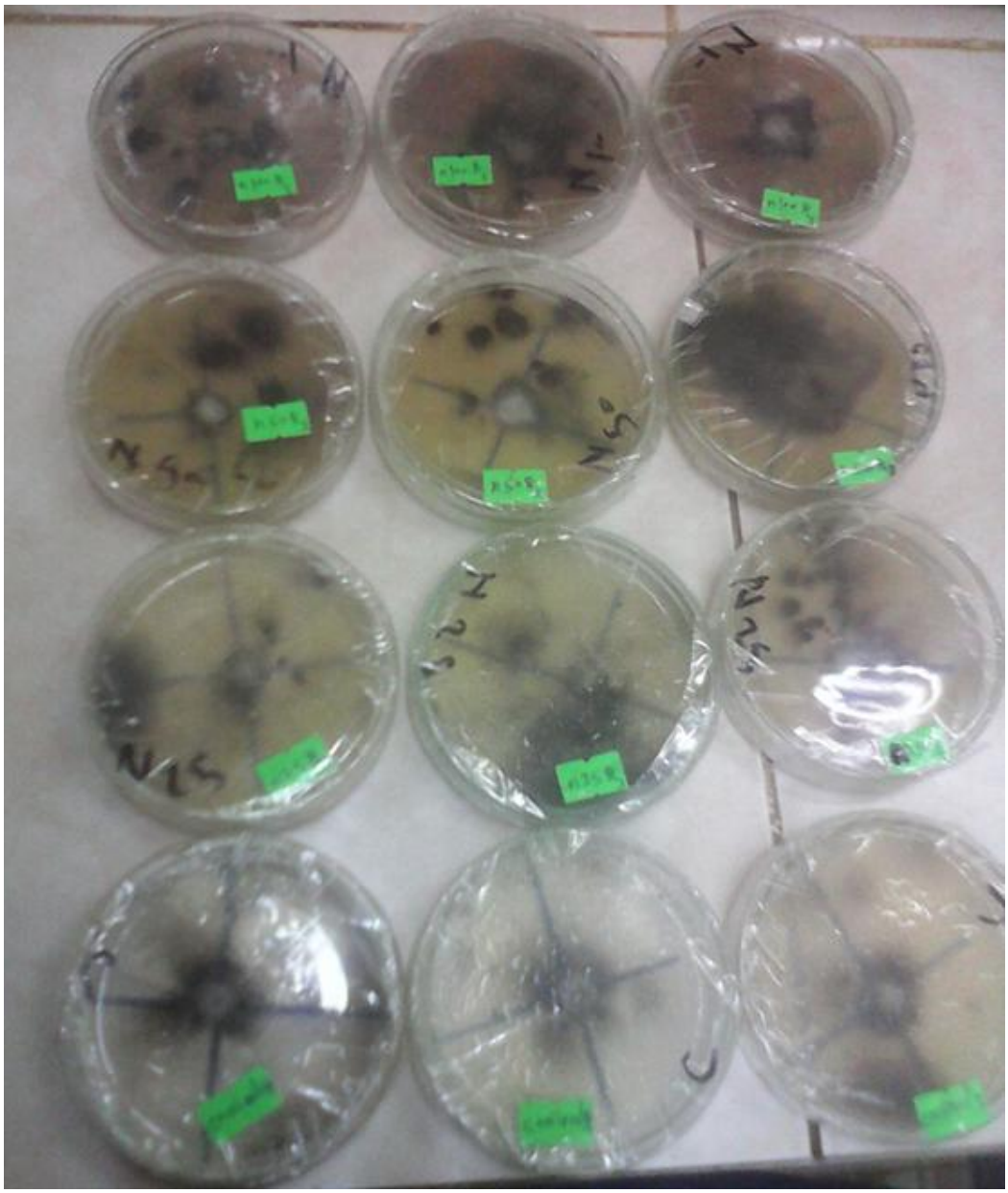
Nelson, T.A. Toussoun and R.J. Cook, Eds. The Pennsylvania State University Press, University Park.

Summeral BA, Salih B, Leslie JF (2003). A utilitarian approach to Fusarium identification. Plant Dis 87:117–128.

Sutherland, J.B., Pometto, A.L. III and Crawford, D.L. 1983. Lignocelluloses degradation by Fusarium species. Can. J. Bot. 61:1194-1198.

Wu, T. L., & Larsen, K. (2000). Zingiberaceae. Flora of China, 24, 322-377.

Images



Appendices

Data File:ALI2016
Title: 2016

Case Range: 16 - 20
Variable 3: first
Function: RANGE

Error Mean Square = 0.1200
Error Degrees of Freedom = 10
No. of observations to calculate a mean = 3

Duncan's Multiple Range Test
LSD value = 0.6302
s_ = 0.2000 at alpha = 0.050
X

Original Order				Ranked Order			
Mean	1 =	6.433	C	Mean	4 =	10.00	A
Mean	2 =	7.967	B	Mean	3 =	9.467	A
Mean	3 =	9.467	A	Mean	2 =	7.967	B
Mean	4 =	10.00	A	Mean	1 =	6.433	C
Mean	5 =	0.7000	D	Mean	5 =	0.7000	D

Data File: _ALI2016_
Title: 2016

Case Range: 16 - 20
Variable 4: second
Function: _RANGE_

Error Mean Square = 0.9520
Error Degrees of Freedom = 10
No. of observations to calculate a mean = 3

Duncan's Multiple Range Test
LSD value = 1.775
s_ = 0.5633 at alpha = 0.050
X
_

Original Order				Ranked Order			
Mean	1 =	6.433	B	Mean	4 =	10.00	A
Mean	2 =	7.667	B	Mean	3 =	9.467	A
Mean	3 =	9.467	A	Mean	2 =	7.667	B
Mean	4 =	10.00	A	Mean	1 =	6.433	B
Mean	5 =	0.7000	C	Mean	5 =	0.7000	C

_

Data File: _ALI2016_
Title: 2016

Case Range: 16 - 20
Variable 5: third
Function: _RANGE_

Error Mean Square = 3.235
Error Degrees of Freedom = 10
No. of observations to calculate a mean = 3

Duncan's Multiple Range Test
LSD value = 3.272
s_ = 1.038 at alpha = 0.050
x
_

Original Order				Ranked Order			
Mean	1 =	4.800	B	Mean	4 =	10.00	A
Mean	2 =	7.267	AB	Mean	3 =	8.500	A
Mean	3 =	8.500	A	Mean	2 =	7.267	AB
Mean	4 =	10.00	A	Mean	1 =	4.800	B
Mean	5 =	0.7000	C	Mean	5 =	0.7000	C

_

Data File : _ALI2016_
Title : 2016

Case Range : 16 - 20
Variable 6 : fourth
Function : _RANGE_

Error Mean Square = 1.621
Error Degrees of Freedom = 10
No. of observations to calculate a mean = 3

Duncan's Multiple Range Test
LSD value = 2.316
s_ = 0.7351 at alpha = 0.050
x
_

Original Order				Ranked Order			
Mean	1 =	5.133	C	Mean	4 =	10.00	A
Mean	2 =	6.300	BC	Mean	3 =	7.733	AB
Mean	3 =	7.733	AB	Mean	2 =	6.300	BC
Mean	4 =	10.00	A	Mean	1 =	5.133	C
Mean	5 =	0.7000	D	Mean	5 =	0.7000	D

_

Data File : _ALI2016_
Title : 2016

Case Range : 16 - 20
Variable 7 : fifth
Function : _RANGE_

Error Mean Square = 0.8450
Error Degrees of Freedom = 10
No. of observations to calculate a mean = 3

Duncan's Multiple Range Test
LSD value = 1.672
s_ = 0.5307 at alpha = 0.050
x
_

Original Order				Ranked Order			
Mean	1 =	6.300	B	Mean	4 =	10.00	A
Mean	2 =	6.967	B	Mean	3 =	7.533	B
Mean	3 =	7.533	B	Mean	2 =	6.967	B
Mean	4 =	10.00	A	Mean	1 =	6.300	B
Mean	5 =	0.7000	C	Mean	5 =	0.7000	C

_

Data File : _ALI2016_
Title : 2016

Case Range : 16 - 20
Variable 8 : ninth
Function : _RANGE_

Error Mean Square = 0.8050
Error Degrees of Freedom = 10
No. of observations to calculate a mean = 3

Duncan's Multiple Range Test
LSD value = 1.632
s_ = 0.5180 at alpha = 0.050
x
_

Original Order				Ranked Order			
Mean	1 =	5.000	C	Mean	4 =	10.00	A
Mean	2 =	5.767	BC	Mean	3 =	7.367	B
Mean	3 =	7.367	B	Mean	2 =	5.767	BC
Mean	4 =	10.00	A	Mean	1 =	5.000	C
Mean	5 =	0.7000	D	Mean	5 =	0.7000	D

_

Data File : _ALI2016_
Title : 2016

Case Range : 16 - 20
Variable 9 : tenth
Function : _RANGE_

Error Mean Square = 0.4850
Error Degrees of Freedom = 10
No. of observations to calculate a mean = 3

Duncan's Multiple Range Test
LSD value = 1.267
s_ = 0.4021 at alpha = 0.050
x
_

Original Order				Ranked Order			
Mean	1 =	5.633	C	Mean	4 =	10.00	A
Mean	2 =	5.533	C	Mean	3 =	7.667	B
Mean	3 =	7.667	B	Mean	1 =	5.633	C
Mean	4 =	10.00	A	Mean	2 =	5.533	C
Mean	5 =	0.7000	D	Mean	5 =	0.7000	D

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Data File : _ALI2016_
Title : 2016

Case Range : 16 - 20
Variable 10 : eleventh
Function : _RANGE_

Error Mean Square = 1.876
Error Degrees of Freedom = 10
No. of observations to calculate a mean = 3

Duncan's Multiple Range Test
LSD value = 2.492
s_ = 0.7908 at alpha = 0.050
x
_

Original Order				Ranked Order			
Mean	1 =	3.767	B	Mean	4 =	10.00	A
Mean	2 =	4.567	B	Mean	3 =	7.733	A
Mean	3 =	7.733	A	Mean	2 =	4.567	B
Mean	4 =	10.00	A	Mean	1 =	3.767	B
Mean	5 =	0.7000	C	Mean	5 =	0.7000	C

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Data File : _ALI2016_
Title : 2016

Case Range : 16 - 20
Variable 11 :tewelveth
Function : _RANGE_

Error Mean Square = 0.5910
Error Degrees of Freedom = 10
No. of observations to calculate a mean = 3

Duncan's Multiple Range Test
LSD value = 1.399
s_ = 0.4438 at alpha = 0.050
x
_

Original Order				Ranked Order			
Mean	1 =	4.300	C	Mean	4 =	10.00	A
Mean	2 =	5.133	C	Mean	3 =	7.767	B
Mean	3 =	7.767	B	Mean	2 =	5.133	C
Mean	4 =	10.00	A	Mean	1 =	4.300	C
Mean	5 =	0.7000	D	Mean	5 =	0.7000	D

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Data File : _ALI2016_
Title : 2016

Case Range : 16 - 20
Variable 12 : thirteenth
Function : _RANGE_

Error Mean Square = 0.7970
Error Degrees of Freedom = 10
No. of observations to calculate a mean = 3

Duncan's Multiple Range Test
LSD value = 1.624
s_ = 0.5154 at alpha = 0.050
x
_

Original Order				Ranked Order			
Mean	1 = 4.700	C		Mean	4 = 10.00	A	
Mean	2 = 5.233	C		Mean	3 = 7.633	B	
Mean	3 = 7.633	B		Mean	2 = 5.233	C	
Mean	4 = 10.00	A		Mean	1 = 4.700	C	
Mean	5 = 0.7000		D	Mean	5 = 0.7000		D

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Data File : _ALI2016_
Title : 2016

Case Range : 16 - 20
Variable 13 : seventeenth
Function : _RANGE_

Error Mean Square = 1.718
Error Degrees of Freedom = 10
No. of observations to calculate a mean = 3

Duncan's Multiple Range Test
LSD value = 2.385
s_ = 0.7567 at alpha = 0.050
x
_

Original Order				Ranked Order			
Mean	1 =	5.467	B	Mean	4 =	10.00	A
Mean	2 =	3.733	B	Mean	3 =	7.933	A
Mean	3 =	7.933	A	Mean	1 =	5.467	B
Mean	4 =	10.00	A	Mean	2 =	3.733	B
Mean	5 =	0.7000	C	Mean	5 =	0.7000	C

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