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Effect of Mesquite aqueous extract and fungicide fulldazin on fungal growth of *Penicillium digitatum*

تاثير المستخلص المائي لاوراق المسكيت والمبيد الفطري فولدازين علي نمو الفطر(بنسليوم ديجيتاتم)

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الآية

(وَهُوَ الَّذِي أَنشَأَ جَنَّاتٍ مَّعْرُوشَاتٍ وَغَيْرَ مَعْرُوشَاتٍ وَالنَّخْلَ وَالزَّرْعَ مُخْتَلِفًا أُكُلُهُ وَالزَّيْتُونَ وَالرُّمَّانَ مُتَشَابِهًا وَغَيْرَ مُتَشَابِهٍ كُلُوا مِن ثَمَرِهِ إِذَا أَثْمَرَ وَآتُوا حَقَّهُ يَوْمَ حَصَادِهِ وَلَا تُسْرِفُوا إِنَّهُ لَا يُحِبُّ الْمُسْرِفِينَ)

سورة الأنعام الآية(141)

DEDICATION

To my mother

To my father

To my brothers and sisters

To all my family, my teachers, colleagues and friends with love and respect.

Gheffar

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All thanks are due to Almighty Allah who gave me health and strength, and helped me tremendously to produce this work.

I would like to express my thanks to my supervisor Dr.Mawahib Ahmed El Siddig, College of Agricultural studies, Sudan University of science and technology, who devoted most of his time to teach us on various disciplines of scientific research.

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ABSTRACT

This study was carried out in the laboratory of plant pathology, plant protection department, college of Agricultural studies, Sudan University of science and Technology in 2017. The objective of this study is to evaluate the effect of Mesquite leaves aqueous extracts and Fulldazin fungicides against Penicillium digitatum in potato tuber.

The aqueous extract prepared of Mesquite leaves, used three concentrations (25%, 50%, and 100%).

Results that have been obtained show that the effect of the aqueous extracts of the leaves Mesquite in all concentrations was of significant effect in inhibiting the growth of fungus compared to the control.

As a result, this study shows that the Mesquite leaves containing materials with the effect of an anti-fungal growth.

ملخص البحث

اجريت هذه الدراسه في مختبر علم امراض النبات , قسم وقاية النبات , كلية الدراسات الزراعية , جامعة السودان للعلوم و التكنولوجيا 2017 لتقييم تأثير المستخلص المائي لأوراق نبات المسكيت علي فطر البنسليوم ديجتاتيم في بيئة بطاطس دكستروز أجار مقارنه بمبيد فيلدازين تحت ظروف المعمل . تم تحضير المستخلص المائي من أوراق المسكيت .استخدمت ثلاثة تركيزات من المستخلص المائي لأوراق المسكيت(25%,50%,100%)النتائج التي تم الحصول عليها في اليوم الثاني والثالث والرابع توضح أن تأثير المستخلصات المائية في أوراق المسكيت في كل التركيزات خلال ثلاث أيام كانت ذات تأثير معنوي في تثبيط نمو الفطر مقارنه بالكنترول.

نتيجة لذلك توضح الدراسة أن نبات المسكيت يحتوي علي مواد ذات تأثير مضاد لنمو الفطر.

CHAPTER: ONE

INTRODUCTION

Mesquite plants, Prosopis juliflora Swartz, belong to family: Leguminosae, it is an invasive, evergreen and multi-purpose leguminous, tree or shrub (Babiker, 2006). The plant is considered native to semi-arid areas of the West Indies Mexico, Central America a northern South America (Felker et al., 2003).

At it is enters of origins the mesquite has played an important social role. In addition to its role in combating desertification and supply of high-value mechanical wood product's fire wood and charcoal mesquite provides shelters, animal feed and food for humans in areas where protein intake is very low and Ander adverse condition of drought and famines (Ibrahim, 1989).

In Sudan, mesquite was introduced in 1917 with the purpose to solve the problem of desertification in some regions in Sudan from South Africa and Egypt (Elamin, 1990), and unfortunately, due to its deliberate distribution within Sudan, the plant became a threat to agriculture and biodiversity. (Babiker, 2006).

Penicillium as well is a large genus containing 150 recognized species, of which 50 or more occur commonly. Many species of Penicillium are isolated from foods causing spoilage. It can cause many diseases in crops, like Green mold.

Fungicide Fulldazin is wettable powder (W.P) 50% Carbendazim

Chemical name: Methyl benzimidazol-2-Ylcarbamate.

CHAPTER: TWO

Literature Review

2-1: Mesquite:

The name Prosopis was selected by Linnaeus to describe the only species he was aware of, p.spicigera, in 1767 (Felker, *et.al*. 2001). Felkeret. Al., (2001) stated that genus Prosopis Linnaeus emends Burk art is in the family leguminosae (Fabaceae), sub-family Mimosoideae. The placing of Prosopis in the wider taxonomic classification system is given below, based on Elias (1981) and Lewis and alias (1981):

2-1-1: Classification:

Family: Leguminosae 650 genera, 18,000 species

Sub-family: Mimosoideae 50-60 genera, 650-725 species

Tribe: Mimoseae 5 tribes

Group: Prosopis 9 groups

Genus: Prosopis 4 genera

2-1-2: Description:

Common mesquite (Prosopis juliflora) a Fabaceae, is an evergreen multi-purpose tree or shrub. Depending on water availability the plant grows up to 12m high or into a shrub. Mesquite growth is not limited by soil type, pH, salinity and/or soil fertility. The tree is a nitrogen fixer, endowed with an extensive root system. Its tap root grows down to 53m and its lateral roots may extend beyond the crown (Choge and Chikamai, 2003). The tree is competitive and allopathic. It is also a prolific seed producer. The seeds, mainly distributed by animals and water, are persistent and a high seed bank often builds up in soil (fowler, 1998).

2-1-3: Economic and other uses:

Various Prosopis species have been introduced to Africa over the past 190 years for their beneficial qualities which include erosion control, shade, fuel wood, building materials, and pods for animal and human consumption in arid and semi-arid regions. The fact that there is clear economic use to this species but severe negative consequences of P. juliflora invasion makes this conflict of interesting species sacrificed (Elsiddig, 2004).

2-1-4: Use of mesquites in biological control:

The results of the effect of aqueous extracts from different parts of Juliflora on the final germination Percentages of seed of Alfalfa crop after four days from sawing. Generally, the result showed that aqueous extract of different parts of mesquite screened, invariably and significantly inhibited the seeds germination of the test crop compared to control where germination is 100%. The inhibitory effect resulted in germination Percentages ranged from 0.0% to 80%.Among different parts of mesquite extract that of fruits and leaves reduced significantly and consistently the seeds germination of test crop. Moreover, the suppressing effect of fruits extract was more pronounced on seeds of Alfalfa than other parts of mesquite (Ahmed, 2016).

2-1-5: Mesquite in Sudan:

Mesquite was introduced into several countries with the primary objective of curbing desertification and providing fire wood and thus preserving indigenous trees (Babiker, 2006; Chog and Chikamai' 2006). However, in most of the countries, where it was introduced,

mesquite has spread outside where it was originally planted and has become a serious weed (El Houri, 1986; Babiker, 1976).

2-1-6: Prevention and control:

2-1-6-1: Cultural control:

High value, such as for agriculture or where labor is relatively cheap. Hand clearing can also be used in conjunction with some mechanical or chemical methods, such as chemical stump treatment (khan, 1961). Grubbing was is more cost effective in lighter infestations. Fire, probably one of the original management tools used in American grassland, has undergone limited assessment for controlling Mesquite.

Young seedlings are sensitive to fire but older trees become hand clearance is the first but method used to deal with Prosopis as awed. Work teams are sent into invaded pasture to fell the trees and uproot all stumps. Although very effective, fire can be used successfully as management tool for preventing the re-establishment of young Prosopis seedling while also improving forage production. Fire has been used in conjunction with other methods in the development of integrated eradication programmers.

Studies on succession suggest the possibility of ecological control, by leaving succession to take its natural course. The invasion of Prosopis species into rangeland has been observed and studied for over century in the USA (Archer, 1995) and for long periods in South America (Antoni and solbring, 1977) and India (chinnimani, 1998).

2-1-6-2: Mechanical control:

Mechanical site cleanse involves tractor operations developed for removing trees in which the roots are severed below ground level to ensure tree kill. These operations include root plunging and changing which are often the most effective mechanical means, using

moldboard plough pulled behind a caterpillar tractor or a heavy chain pulled between tow machines. For root ploughing, large trees must first be felled by hand, but this treatment has been used to remove stumps up to 50cm in diameter without difficulty and has treatment life of 20 years or more (Jacoby and Ansley, 1991).

2-1-6-3: Chemical control:

Chemical treatments involve the use of herbicide to kill trees, with the most effective being stem or aerial applications of systemic herbicides. Effectiveness is dependent upon chemical uptake, which in Prosopis is limited by the thick bark, woody stems and small leaves with a protective waxy outer layer difficult. Many herbicides and herbicide mixtures have been tested, mostly on P.glandulosa until the banning of its use in the 1980s, 2,4,5-T was the herbicide of choice in the USA (Jacoby and Ansley, 1991). And Australia (Csurhes, 1996).Although 2, 4-D provided excellent suppression of top growth, few trees were actually killed and such chemical treatments had to applied periodically to ensure that forage yields were maintained.

Infested sites often needed spraying ever 5-7 years. The most effective chemical for high tree kill in the USA is clopralid, but dicamba, picloram and triclopyr have also been successfully used, either alone or in combination (Jacoby and Ansely, 1991). In India, ammonium sulfa mate was successful in killing P.juliflora trees and as a stump treatment (paschal and Shetty, 1977).

2-1-6-4: Biological control:

Several biological control programmers using species of seed-feeding brushed beetles have been developed and implemented. The Advantages With brushed is their observed host specificity with many species found to feed only on Prosopis, and some only on a single species. Other insect species known to have deleterious effect

On native and exotic Prosopis in The Americas, mainly twig girdlers and psyllids have also been suggested as possible biological control agents.

The same tow brushed species were also introduced to Ascension Island in an attempt to control P.juliflora which is present on 80% of theisland, often in dance thickets. Two other species, one a psyllids and the other amirid, were identified as attaching Juliflora on Ascension Island and were thought to have been introduced accidentally from the car bean. The mired Rhizocloa sp. Causes widespread damage and is thought to lead to substantial mortality of trees (fowler, 1998).In Australia, Prosopis infestation are at a relatively early stage and extreme care is being employed in the selection of suitable biological control agents, following the long history of problems caused there by plant and animal introduction. Insect species continue to be tested for their efficacy and host specificity as possible biological control agents of Prosopis species in Australia (e.g. Van klinken, 1999, .van klinken *etal*., 2009).

Prosopis species continue to spread widely imparts of their native ranges where many insect species including brushed, spilled and other injurious pests are common components of the ecology. These regularly attack Prosopis but the trees have adapted to infestation by these pests and are still able to become invasive weeds over large tracts of land (Venklinhen, 1999).

2-1-6-5: Integrated control:

Fire has been used in conjunction with other methods in the development of integrated eradication programmers. For example spraying with herbicides produces dead wood that will ignite and support a sustained fire with more likelihood of killing the remaining trees.

Prosopis juliflora is retorted to influence the growth of other plants. Phenolic compounds present in this plant have biological toxicity towards many plants and can cause disturbances in various processes by interfering with the enzymology of the target plants (Rizvi and Rizvi, 1992. Thoyabet *et al*., 2009). Thus, mesquite plant is an invasive species which widespread in many countries. Shankhla *et al*., (1965) reported the inhibitory effect of *Prosopis juliflora* aqueous extracts on the growth of some plants. Similar results were reported by many workers (Nakano *et al.,* 2001, Noor *et al*., 1965., Al-Humid and Warrag, 1997., Warrag, 1995).

2-2: *Penicillium spp:*

Penicillium as well is a large genus containing 150 recognized species, of which 50 or more occur commonly. Many species of Penicillium are isolated from foods causing spoilage; in addition, some may produce bioactive compounds. Important mycotoxin produced by Penicillium includes ochratoxin A, patulin, citrinin and penitrem A. Some of the most important toxigenic species in foods are *Penicillium expansum, Penicillium citrinum, Penicillium crustosum and Penicillium verrucosum*. A much larger number of Penicillium species are mainly associated with food spoilage. Those include *Penicillium aurantiogriseum, Penicillium chrysogenum, Penicillium digitatum, Penicillium griseofulvum, Penicillium italicum, Penicillium oxalicum* and *Penicillium viridicatum*; some of these produce mycotoxins. However, *Penicillium* species are associated more with cool temperate and temperate crops, mainly cereals, since most species do not grow very well above 25-30°C (Pitt , 2006).

2-2-1: Disease caused by *Penicillium*:

Green mold in citrus:

Green mold caused by Penicillium digitatum (Pers.) Sac .Causes significant losses of CV. Satsuma mandarin (Citrus reticulate Blanco) after harvest in New Zealand. Traditionally, green mold has been controlled by postharvest application of fungicides (Eckert & Eaks 989; Brown & Wardowski 984; Tavernier 200).

2-3: Potato plant:

2-3-1: Economic importance of potato:

The potato is a starchy tuberous crop from the perennial Solanum tuberosum of the solanaceae family (also known as the nightshades).

The word potato may refer to the plant itself as the edible tuber.

One of the major constraints facing the quantity, quality and availability of healthy crop worldwide are the losses and contamination caused by post-harvest diseases .The major groups of postharvest disease are those which arise from infections initiated during and after harvest.

The threat to potatoes from fungal infections has now reached a level that outstrips that posed by bacterial and viral diseases (Berger, 1977). One of the main fungal pathogens that attack potatoes is Fusarium spp which are a worldwide economic problem (Nielson, 1981).

The disease affect tubers in storage and seed potato pieces after planting late Blight ,Early Blight ,Black scurf ,dry rot green mold.

In the region of the Andes, there are some to her closely related cultivated potato species.

Potatoes are the world's fourth largest food crop, following rice, wheat, and maize.

Longs –term storage of potatoes requires specialized care in cold warehouses and such warehouses are among the oldest and largest storage facilities for perishable goods in the world.

Once established in Europe, the potato soon became an important food staple and field crop.

The annual diet on of an average global citizen in the first decade of the twenty-first century included about 33Kg (or 73 IB) of potato.

However, the local importance of potato is extremely variable and rapidly changing.

It remains an essential crop in Europe, where per capita production is still the highest in the world, but the most rapid expansion over the past few decades has occurred in southern and eastern Asia.

China is now the world's largest potato-producing country, and nearly a third of the world's potatoes are harvested in China and India (Thompson and Morgan, 1855).

CHAPTER: THREE

MATERIALS AND METHODS

3-1: Experimental site:

Experiments were carried out in the laboratory of plant pathology, department of plant protection, college of Agricultural Studies, Sudan University of Science and Technology during August 2017.

3-2: Collection of mesquite plant:

The leaves of mesquite were collected from trees growing in the premises of the college of Agricultural Studies, Shambat. The parts collected were cleaned from dust and material by hand, washed with distilled water, surface sterilized with 5% ethanol Alcohol, thoroughly washed in sterilized water and dried shade at ambient temperature, ground and powdered separately to obtain fine powder for extraction and kept till use.

3-3: Preparation of aqueous extract of mesquite leaves plant parts:

Aqueous extracts of each of the mesquite parts were prepared as recommended by Okigbo (2006). The obtained fine powder form different parts of mesquite was weighted (500gm) and added to it 1000 ml sterilized distilled water and then placed in shaker for 24 hrs. The extracts were filtered using what man No.1 filter paper and in the refrigerator to serve as stock solution (plate1).

3-4:Fungicide process:

The chemical tested were fulldazin fungicides 10ml dissolved in 100ml of sterilized distilled water to give 5, 10, 15 ppm respectively . For this solution 5, 10, 15 were completed to 100ml by adding sterilized potato dextrose agar medium to give final concentration

Plate 1: The aqueous extracts of mesquite leaves.

3-5:Isolation of Penicillium digitatum from plant material:

The Infected potato tubers showing symptom of green mold disease were collected from local market in Khartoum and cut into small pieces(0.5, 1.0 cm), washed thoroughly with tap water, sterilized with Clorox (NaOCI) (1%) for 1 minute, rinsed three time in sterilized distilled water and dried on sterilized filter papers. The sterilized section were thenseededat potato dextrose agar medium (PDA) in petri dishes' (9cm), 6 pieces/ per plate.

The inoculated petri dishes were incubated at $25C^0$ for 7 days. After incubation, growing fungi were sub cultured on PDA medium for further purification of the fungus. Furthermore, Compound

microscopic examinations were carried out for Mycelia and Conidia structure based on the method of booth, (1977) to confirm that the fungus is *Penicillium digitatum.*

Identification of the fungus was supplemented by already prepared by slides of *P.digitatum* at the pathology laboratory. Standard books and research papers were also consulted during the examination of this fungus (Aneja, 2004). The purified isolates were maintained on PDA medium for further studies.

3-6:Data analysis:

All the data were determined by Analysis of Variance (ANOVA) using a completely randomized design. The significance of differences between treatments were determined, using the Duncan's Multiple (DMR)test of Statistical Analysis System.

CHAPTER FOUR

RESULTS AND DISCUSSION

4-1: Results:

This study were conducted under laboratory condition plant pathology, Department of plant protection, college of Agricultural studied, Sudan University of science and Technology during the period August to October 2017 to determine the inhibitory effect of mesquite leaves aqueous extracts and fungicide, Fulldazin 50wp against the fungus *Penicillium digitatum*.

The results in Table 1 Figure 1, show the mesquite leaves aqueous extract tested and fungicide fulldazin top to growth of *Penicillium digitatum.* All concentration of mesquite had on effects on the fungal growth three days post inoculation. In the fourth day there are no significant differences between 50%, 100% concentrations of mesquite and control , while the Fulldazin 50wp fungicide and concentration 25% recorded(7.9and 0) in linear growth of the fungus.

After four days post inoculation the results in Table 2 show the inhibition zone of mesquite leaves aqueous extract and fungicide in fungus growth. The concentrations 100%, 50% 25% gave (18.4, 40.6 and 16.3 respectively). On the other hand Fulldazin 50wp fungicide gave the highest inhibition zone (100%) Obviously, the test organism and fungicide differs in its response to the different concentrations but on the whole, growth inhibition increased with the increasing concentration.

Table(1): Effect of aqueous extract of mesquite leaves, (*Prosopis spp*) and fungicide fulldazin 50wp top on the linear growth *of Penicillium digitatum* (two, three and four days) post inoculation

Mesquite extract concentration	Linear growth(cm)		
	2days	3days	4days
25%	(7.4)a	(7.8)e	(7.9)e
50%	(3.8)ab	(5.4)f	(7.4) ab
100%	(6.7)c	(7.9)ab	8abc
Control	(9.2)a	(9.6)ab	(9.8)a
Fungicides	(0.0)a	(0.0)a	(0.0)d
C.V	28.6%	19.1%	22.3%
L.S.D	4.442	1.714	3.788

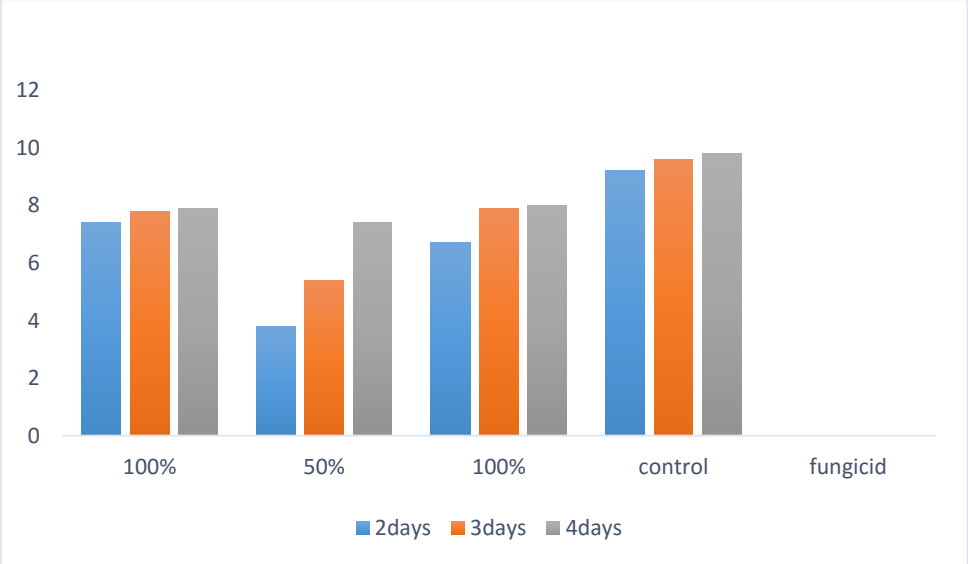

Figure1:**Effect of aqueous extract of mesquite leaves, (*Prosopis spp*) and fungicide fulldazin 50WP top on the linear growth *of Penicillium digitatum* (two, three and four days) post incolation**

Table(2):Effect of aqueous extract of mesquite leaves, (*Prosopis spp*) and fungicide fulldazin 50WP top on the fungal growth of *Penicillium digitatum* five days post inoculation.

Agues mesquite leaves extract Concentration	% Reduction in fungus linear growth
25%	16.3
50%	40.2
100%	18.4
Fungicide	100
Control	0

DISCUSSION:

The results revealed that plants extracts had a strong antifungal activity with significant inhibition on the growth of the fungus *Penicillium digitatum*. Aqueous extract of mesquite leaves, (*Prosopis spp)* was the most effective to inhibit the growth of the tested fungus. On the other hand, the chemical fungicide Fulldazin 50wp was more efficient than natural compounds.

The results similar of this study corresponds with work done by William(2008) who reported that sprays made from aqueous garlic extracts have antibiotic and antifungal properties and will suppress a number of plant diseases, including powdery mildew on cucumbers and to some extent black spot on roses. Similar results were reported by Slusarenko *et al.* (2008). Also the results is agree with scientists who reported that the mycelia growth of various species of *Penicillium* was inhibited by the plant extracts of *Allium cepa* (Patel, 1989), *Cassia nodosa(*Reddy and Reddy, 1987); *Azadirachta indicia* (Eswaramoorthy *et al*, 1989);*Allium sativum* and *Sapindus trifoliate* (Gohil and Vala, 1996); Neem seed extract (Gour and Sharmaik, 1998),*Eucalyptus amygdalina*, (Bansal and Gupta, 2000).

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FAOSTAT data, 2006 important crop worldwide and ranks Fourth in production potato among food crops after Maize (*Zea Mays* L.), rice (*Oryza sativa* L.), And wheat |(*Tritium aesitvum* L.)

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