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IN-VIVO CONTROL OF *FUSARIUM MONILIFORME* CAUSING ROOT ROT OF JATROPHA THROUGH CHEMICAL AND AQUEOUS PLANT EXTRACTS

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ABSTRACT

Jatropha (*Jatropha curcas*) is a plant of the Euphorbiaceae family that grows fast and able to be cultivated in tropical and sub-tropical regions. It is also known as Jamalghota and Jangli in different regions of the world and reported to be grown along with other crops such as wheat, sunflower, cluster bean, and pearl millet.Jatropha seeds were collected from the field of a project run by the Department of Agronomy, Sindh Agriculture University, Tando Jam. In the isolation process, seeds of jatropha were surface sterilized in a 5% bleach solution for 1-2 minutes to remove contamination.The experiment was carried in nursery bags to seethe effect of different doses of plant extracts on root rot of jatropha. The aqueous extract of different plant species was prepared by grinding freshly collected leaves of Neem, Ginger, Basil, Acasia, Garlic, Toothbrush plant, Eucalyptus, Giant milkweed, Mint, and Aloe vera separately. These extracts were then stained with a muslin cloth and a piqued solution was stored. Later it was used at the dose of 10ml, 20ml, and 30ml per bag containing disease-free (sterilized in soil sterilizer) soil. For comparison, two treatments (untreated, inoculated, and untreated, un-inoculated) bags were placed.The results of the experiment showed a significant growth of shoot length and weight in inoculated plants treated with the highest dose of Aliette, followed by Carbendazim, and Metalyxl+Mancozeb. Whereas minimum shoot length and weight was observed in control. Similarly, maximum root length and weight were recorded in plants treated with Aliette, followed by Carbendazim and Metalyxl+Mancozeb as compared to control.

Key Words: *Fusarium moniliforme*, Jatropha,Control, Plant extracts, Root rot

INTRODUCTION

Jatropha (*Jatropha curcas),* a fast-growing plant of the Euphorbiaceae family also known as Jamalghota, Jangliarandi in different parts of the world is grown in tropical areas and subtropical areas on average to wastelands. Native region to Jatropha South America later it traveled to African and other regions of the world. It tells that the Portuguese took it and grown in Africa and Asia. It is a shrub, hard in nature, can grow and develop in dry weather, and is not fed by animals. It can thrive well on stony, gravelly, shallow, degraded, and salt-affected soils having low fertility and moisture content. Jatropha can grow easily from seeds nursery and stem cutting. It is reported to be grown along with other crops such as wheat, sunflower, cluster bean, and pearl millet. Jatropha produces seeds that are rich in oil, it has proved that the oil produced from Jatropha seed is a commercially viable source of renewable energy. In this regard, several studies have been carried around the world that prove that Jatropha oil is potent to be used as an alternative source diesel engine. The yield of Jatropha varies with practices done but on average it yields 1892 liters of oil. In the process of its growth, development, and production that plants absorb carbon from the environment and gives Oxygen, thus the oil produced by it is called environment friendly and biofuel. It removes Carbon from the environment by storing it in its tissues and converting it into soil carbon. Other than biodiesel, its uses are conspicuous. Jatropha seeds are rich in Nitrogen, Phosphorus, and Potash 4.44%, 1.4%, and 1.2% respectively thus used as manure of crops. these are also used as a lubricant, in soap making process and candle manufacturing. (Punia, 2007; Kureel *et al.,* 2007; Agro Forestry Tree Database, 2007; Henning, 2011). Above all Jatropha has also medicinal importance in human life. Like other shrubs, its use as traditional medicine and veterinary purpose can't be denied. Use of decoction of leaves, stem bark, sapwood, and green leaves themselves are effective to arrest bleeding of wounds, remediate cough, solve skin problems and soothe pain caused by inflammation and pain in the joints and muscles. Having anti-mollusks properties Jatropha seeds are effectively used against various species of snails. Its use at the dose of 100 ppm against gram-positive and gram-negative (*Staphylococcus* spp. and *Pseudomonas* spp.) respectively, through agar well diffusion and agar dilution serial method has successfully proved that Jatropha also has viable anti-bacterial properties. The experiment conducted in this regard showed that extracts significantly exhibited their antibacterial activities with different extents (Joachim, 1996; Batugal *et al.,* 2004; Sutthiduen, 2007). Damping-off (*Fusarium moniliforme*), Dry root rot (*Macrophominaphaseolina*), Leaf spot *(Alternariasp. & Cercospora sp.*), Powdery mildew (*Oidiopsissp),* Collar or

root rot (*Sclerotium rolsfii),* Fruit rot disease, Stem canker, and Mosaic virus are the diseases of Jatropha caused by various pathogenic groups are prevailing in different localities of India where jatropha is grown (Punia, *et al.,* 2010).

MATERIAL AND METHODS

Sample collection and isolation

For isolation of the pathogen, jatropha seeds were collected from the field of a project run by the Department of Agronomy, Sindh Agriculture University, Tando Jam. In the isolation process, seeds of jatropha were surface sterilized in a 5% bleach solution for 1-2 minutes to remove contamination. Then seeds were triple rinsed with distilled water to eliminate remains of bleach. The seeds were dried on blotting paper to avoid the moisture from the seed surface. To isolate the pathogen from the roots of a plant was having symptoms of root rot was uprooted and brought to the laboratory. Its root portion was washed with tap water then roots were dried on blotting paper. The infected root portion was cut into pieces of ½ inch with the help of scissors and surface sterilized in a 5% bleach solution for 30 seconds. Treated root pieces were given a triple rinse with distilled water to eliminate remains of bleach and then the root pieces were placed on tissue paper for drying.

Culture of fungus

Each sterilized potato dextrose agar (PDA) medium containing the Petri plate was inoculated with five seeds treated with bleach and triple rinsed with distilled water. Then all Petri dishes were placed in an incubator at a temperature of 25±2℃ for the growth of fungus. Isolation of pathogen was also done by following the same process. The root of the infected plant was cut into small ½ inch pieces a were sterilized in a bleach solution. Then root pieces were rinsed in distilled water for three times. Fresh and sterilized PDA was poured in Petri dishes and root pieces were placed on them. After that inoculated plates were sealed with a plastic tap and kept at 25±2℃ for the growth of fungus. Fungus grown on Petri dishes was purified by growing it on fresh PDA. The identification of Fungus was done based on colony growth, colony color, and morphology of conidia observed under 100 power lenses of the microscope. Isolated fungus was further multiplied on the sterilized potato dextrose agar medium.

Pure culture

Pure culture of *Fusarium moniliforme* was maintained by taking fungus disc with sterilized 6mm cork borer from actively growing mycelium and was placed PDA medium for use in different experiments.

Pathogenicity test

An experiment was designed to ensure the pathogenicity test of *Fusarium moniliforme* causing root rot in jatropha. A diluted solution of 250ml distilled water and one full plate of fungus culture was mixed and shaken well. Plants were grown in a nursery and were maintained for one month. Then one-month-old healthy plant bags were drenched with 20ml of a fungal solution containing 700g sterilized soil. Bags given sterilized water were kept as control. After 30 days root pieces were cultured on sterilized potato dextrose agar medium (PDA) medium to see the growth of fungus from infected tissues of jatropha.

Effect of different plant extracts on the root rot jatropha.

The experiment was carried in nursery bags to see the effect of different doses of plant extracts on root rot of jatropha. The aqueous extract of different plant species was prepared by grinding freshly collected leaves of Neem, Ginger, Basil, Acasia, Garlic, Toothbrush plant, Eucalyptus, Giant milkweed, Mint, and Aloe vera separately. These extracts were then stained with a muslin cloth and a piqued solution was stored. Later it was used at the dose of 10ml, 20ml, and 30ml per bag containing disease-free (sterilized in soil sterilizer) soil. For comparison, two treatments (untreated, inoculated, and untreated, un-inoculated) bags were placed. The bags were put in the design of the randomized complete block, each treatment was replicated four times. After the sowing of seeds, nursery bags were artificially inoculated with an inoculum of *Fusarium moniliforme* at the dose of one petri dish (fully covered of colony growth). Water was applied on a need basis and after 60 days of sowing, disease incidence percent and growth parameters were recorded.

Effect of different fungicides on the root rot of jatropha

An experiment was conducted to check the efficacy of different fungicides against root rot of Jatropha, caused by *Fusarium moniliforme*. Fungicides Aliette, Carbendazim, Metalyxl+Mancozeb, Thiomil, and Clipper were tested against the fungus. The method used for testing fungicides was the seed treatment method. Seeds were treated in the fungicide solution of different doses that were prepared at the rate of 2g, 3g, and 4g of fungicide and 1 liter of distilled water. Nursery bags were filled with sterilized soil and then the soil was artificially infested with fungus inoculum at the rate of 1 plate (fully grown petri dish with mycelium of *Fusarium moniliforme*) per bag. Then the treated seed of jatropha was sown in bags (4 seeds/bag) containing already infested soil. The bags were arranged in a randomized complete block design, each treatment was repeated four times. For getting treatments compared, two treatments of untreated, inoculated (control 1) and untreated; un-inoculated (control 2) were used. Nursery bags were applied with water in uniform quantity at an equal interval. Observation of parameters like disease incidence, shoot length, root length, root weight, and root weight was taken after 60 days of sowing. All the data were statistically analyzed by using ‘student edition of statistics, version 1.0’ computer software for analysis of variance and LSD at 5% to compare the differences among

Table.1 Effect of different plant extracts on disease incidence of root rot of jatropha inoculated with *Fusarium moniliforme*

Plant extracts	Disease incidence			Mean of infected plants	Disease incidence (%) decreased over control
	D1	D2	D3		
Neem	5.50g	4.75g	3.50g	4.58g	54.2
Ginger	6.00fg	5.00g	4.00fg	5.00fg	50.00
Basil	6.25efg	5.50fg	5.00ef	5.58f	44.2
Acasia	6.75ef	6.25ef	6.00de	6.33e	36.7
Garlic	7.25de	7.00de	6.75d	7.00de	30.00
Tooth brush plant	8.00cd	7.75cd	7.00d	7.58d	24.2
Eucalyptus	8.75bc	8.50bc	8.25c	8.5c	15.00
Giant milk weed	9.00abc	8.75bc	8.50bc	8.75bc	12.5
Mint	9.50ab	9.50ab	9.00abc	9.33ab	6.7
Aloe vera	10.00a	10.00a	9.50ab	9.83a	1.7
C1	10.00a	10.00a	10.00a	10.00a	----
C2	0.00h	0.00h	0.00h	0.00h	----
LSD (P=0.05)	1.121	1.121	1.121	0.676	------

C1 = Control (untreated, inoculated), C2 = Control (untreated, un-inoculated)

Table.2: Effect of different plant extracts on plant growth (shoot length and shoot weight) of jatropha inoculated with *Fusarium moniliforme.*

Plant extracts	Shoot length (cm)					Shoot weight (g)				
	D1	D2	D3	C1	C2	D1	D2	D3	C1	C2
Neem	13.00a	14.00a	14.75a	9.00	16.00	2.900a	2.95a	3.00a	1.40	3.30
Ginger	12.75ab	13.00ab	14.00ab	9.00	16.00	2.880a	2.91a	2.95ab	1.40	3.30
Basil	12.50ab	12.75b	13.00bc	9.00	16.00	2.870a	2.90a	2.95ab	1.40	3.30
Acasia	12.00abc	12.25bc	12.50cd	9.00	16.00	2.710ab	2.77a	2.80abc	1.40	3.30
Garlic	11.75bc	12.00bcd	12.25cde	9.00	16.00	2.450abc	2.59ab	2.69abc	1.40	3.30
Tooth brush plant	11.25cd	11.50cde	12.00cdef	9.00	16.00	2.300abc	2.40ab	2.55abc	1.40	3.30
Eucalyptus	11.00cde	11.25cde	11.50def	9.00	16.00	2.000abc	2.10ab	2.18abc	1.40	3.30
Giant milk weed	10.50de	11.00de	11.25ef	9.00	16.00	1.800abc	1.95ab	2.00abc	1.40	3.30
Mint	10.00ef	10.80e	11.00fg	9.00	16.00	1.670bc	1.80ab	1.90bc	1.40	3.30
Aloe vera	9.25 f	9.500f	10.00g	9.00	16.00	1.400c	1.50b	1.86c	1.40	3.30
LSD(P=0.05)	1.179	1.179	1.179	----	----	1.118	1.177	1.079	----	----

C1 = Control (untreated, inoculated), C2 = Control (untreated, un-inoculated)

Table.3: Effect of different plant extracts on plant growth ((root length and root weight) of jatropha inoculated with *Fusarium moniliforme.*

Plant extracts	Root length (cm)					Root weight (g)				
	D1	D2	D3	C1	C2	D1	D2	D3	C1	C2
Neem	5.00a	5.50a	5.75a	3.50	6.75	0.38a	0.39a	0.40a	0.29	0.50
Ginger	5.00a	5.25ab	5.50ab	3.50	6.75	0.37a	0.38a	0.40a	0.29	0.50
Basil	4.75ab	5.00abc	5.25abc	3.50	6.75	0.35b	0.36b	0.38b	0.29	0.50
Acasia	4.50abc	4.50abcd	4.75abcd	3.50	6.75	0.33c	0.34b	0.35c	0.29	0.50
Garlic	4.00abc	4.25bcd	4.50bcd	3.50	6.75	0.32cd	0.33bc	0.34cd	0.29	0.50
Tooth brush plant	4.00abc	4.10bcd	4.30cd	3.50	6.75	0.31de	0.32cd	0.33de	0.29	0.50
Eucalyptus	3.75bc	4.00cd	4.20cd	3.50	6.75	0.30ef	0.31de	0.32ef	0.29	0.50
Giant milk weed	3.75bc	3.90cd	4.10cd	3.50	6.75	0.30ef	0.30e	0.31fg	0.29	0.50
Mint	3.60bc	3.75d	4.00d	3.50	6.75	0.30ef	0.30e	0.31fg	0.29	0.50
Aloe vera	3.50c	3.75d	3.75d	3.50	6.75	0.29f	0.30e	0.30g	0.29	0.50
LSD (P=0.05)	1.201	1.179	1.179	----	----	0.011	0.011	0.011	----	----

C1 = Control (untreated, inoculated), C2 = Control (untreated, un-inoculated)

Table.4 Effect of different fungicides on disease incidence of root rot of jatropha inoculated with Fusarium moniliforme

Plant extracts	Disease incidence			Mean of infected plants	Disease incidence (%) decreased over control
	D1	D2	D3		
Aliette	2.00d	1.00de	0.00d	1.00de	90.00
Carbendazim	3.00d	2.00d	1.00d	2.00d	80.00
Metalyxl+Mancozeb	4.75c	3.50c	2.50c	3.58c	64.20
Thiomil	5.50c	4.50c	3.25c	4.42c	55.80
Clipper	7.00b	6.00b	5.25b	6.08b	39.20
C1	10.00a	10.00a	10.0a	10.00a	----
C2	0.00e	0.00e	0.00d	0.00e	----
LSD (P=0.05)	1.111	1.111	1.014	1.111	----

C1 = Control (untreated, inoculated), C2 = Control (untreated, un-inoculated)

Table.5 Effect of different fungicides on plant growth (shoot length and shoot weight) of jatropha inoculated with *Fusarium moniliforme*

Plant extracts	Shoot length (cm)					Shoot weight (g)				
	D1	D2	D3	C1	C2	D1	D2	D3	C1	C2
Aliette	13.50a	15.00a	16.00a	9.00	16.00	3.00a	3.10a	3.30a	1.40	3.3
Carbendazim	12.70a	14.00a	15.00a	9.00	16.00	2.70ab	2.80ab	3.00a	1.40	3.3
Metalyxl+ Mancozeb	11.00b	12.00b	13.20b	9.00	16.00	2.30ab	2.50ab	2.75a	1.40	3.3
Thiomil	10.50b	11.30bc	12.00bc	9.00	16.00	2.00ab	2.33ab	2.51a	1.40	3.3
Clipper	10.00b	10.50c	11.00c	9.00	16.00	1.66b	1.85b	2.10a	1.40	3.3
LSD (P=0.05)	1.230	1.230	1.230	----	----	1.230	1.230	1.230	----	----

C1 = Control (untreated, inoculated), C2 = Control (untreated, un-inoculated)

Table.6 Effect of different fungicides on plant growth (root length and root weight) of jatropha inoculated with *Fusarium moniliforme.*

Plant extracts	Root length (cm)					Root weight (g)				
	D1	D2	D3	C1	C2	D1	D2	D3	C1	C2
Aliette	6.00a	6.25a	6.75a	3.50	6.75	0.40a	0.45a	0.50a	0.29	0.50
Carbendazim	5.75ab	6.00ab	6.50a	3.50	6.75	0.38a	0.40ab	0.44ab	0.29	0.50
Metalyxl+ Mancozeb	5.00ab	5.50ab	6.00ab	3.50	6.75	0.35ab	0.38b	0.40bc	0.29	0.50
Thiomil	4.75bc	5.00bc	5.25bc	3.50	6.75	0.33ab	0.35bc	0.38bc	0.29	0.50
Clipper	3.75c	4.00c	4.25c	3.50	6.75	0.30b	0.32c	0.34c	0.29	0.50
LSD (P=0.05)	1.230	1.230	1.230	----	----	0.078	0.056	0.078	----	----

C1 = Control (untreated, inoculated), C2 = Control (untreated, un-inoculated)

treatment means.

RESULTS AND DISCUSSION

Isolation

This work carried out for isolation and identification of fungi responsible for root rot of jatropha, pathogenicity test for confirmation of disease, and check-in field conditions effect of moisture and inoculum levels on disease development and the effect of soil amendments, different fungicides, and plant extracts on disease incidence and plant growth of jatropha.

Isolation and identification of the fungus

Fusarium moniliforme was isolated from infected roots and seed of jatropha by growing on the PDA medium. Colony growth of the fungus was fast-growing, initially white later turned in yellowish, reddish, and golden brown. The small hyaline mostly single-celled microconidia were seen in long catenate chains arising from morphologically simple phialides. These characters of the fungus matched with the findings of various scientists reported for *Fusarium moniliforme.*

Effect of plant extracts on root rot of Jatropha caused by *Fusarium moniliforme*

Ten plant species belonging to different families were selected to observe the antifungal activity against *Fusarium moniliforme*with different doses in an *in-vivo* experiment. The results indicated that higher doses of Neem and Ginger extracts significantly decreased the disease incidence (54.2 and 50.00%) respectively as compared to other plants and control. While fungus was poorly sensitive to Aloe vera, Mint, Giant milkweed extracts (Table-1). Maximum shoot length and weight

were seen in inoculated plants treated with a higher dose of Neem (14.75cm, 3.00g) followed by Ginger (14.00cm, 2.95g), Basil (13.00cm, 2.95), and Acacia (12.50cm, 2.80g) as compared to other treatment and control (Table 2). Similarly significantly highest root length and weight was recorded in plants treated with Neem (5.75cm, 0.40g) followed by Ginger (5.50cm, 0.40g), Basil (5.25cm, 0.38g), and Acacia (4.75cm, 0.35g) as compared to other plant extracts and control (Table. 3)

Effect of different fungicides on root rot of Jatropha caused by *Fusarium moniliforme.*

A variable effect of different fungicides and doses was seen for disease incidence and plant growth parameters. The results showed that at a higher dose of Aliette and Carbendazim significantly decreased the disease incidence 90 and 80% respectively, as compared to Metalyxl+Mancozeb (64.20%), Thiomil (55.80), Clipper (39.20%), and control(Table-4).

Significantly maximum shoot length and weight were observed in inoculated plants treated with the highest dose of Aliette (16.00cm, 3.30g) followed by Carbendazim (15.00cm, 3.00g) and Metalyxl+Mancozeb (13.20cm, 2.75g) whereas, minimum shoot length and weight were observed in control (Table-5). Similarly, maximum root length and weight was recorded in plants treated with Aliette (6.75cm, 0.50g) followed by Carbendazim (6.50cm, 0.44g) and Metalyxl+Mancozeb (6.00cm, 0.40g) as compared to control (Table-6).

Discussion

Isolation and identification of the fungus

Fusarium moniliforme was isolated from infected roots and seed of jatropha by growing on the PDA medium. Colony growth of the fungus was fast-growing, initially white later turned in yellowish, reddish, and golden brown. Characteristics of conidia were small in size, hyaline, microconidia connected in long chains arising from morphologically simple phialides. These characters of the fungus matched with the findings of various scientists reported for *Fusarium moniliforme.*

Effect of different fungicides on plant growth (root length, root weight, and shoot length, shoot weight) of Jatropha inoculated with *Fusarium moniliforme.*

The results of the experiment showed a significant growth of shoot length and weight in inoculated plants treated with the highest dose of Aliette (16.00cm, 3.30g) followed by Carbendazim (15.00cm, 3.00g) and Metalyxl+Mancozeb (13.20cm, 2.75g) whereas, minimum shoot length and weight was observed in control. Similarly, maximum root length and weight were recorded in plants treated with Aliette (6.75cm, 0.50g) followed by Carbendazim (6.50cm, 0.44g) and Metalyxl+Mancozeb (6.00cm, 0.40g) as compared to control. Kureel *et al.,* (2007) reported that the application of Carbendazim and other chemicals were effective for better disease control and higher yield of jatropha. Omar *et al.,* (2006);Nikam *et al.,* (2007) also reported that Carbendizm was found effective against *Fusarium spp*.Anonymous, (2011) has reported that Carbendizm is better chemical control of Fusarium in jatropha

Effect of plant extracts on plant growth (root length, root weight, and shoot length, shoot weight) of Jatropha inoculated with *Fusarium moniliforme.*

The experiment showed maximum shoot length and weight in inoculated plants treated with a higher dose of Neem (14.75cm, 3.00g) followed by Ginger (14.00cm, 2.95g), Basil (13.00cm, 2.95), and Acacia (12.50, 2.80g) as compared to other treatment and control. Similarly significantly highest root length and weight were recorded in plants treated with Neem (5.75cm, 0.40g) followed by Ginger (5.50cm, 0.40g), Basil (5.25cm, 0.38g), and Acacia (4.75cm, 0.35g) as compared to other plant extracts and control. Sharma and Trivedi, (2002), Uzma *et al.,* (2008), Hassanein, *et al.,* (2010), Owolade, *et al.,* (2000), and Babu *et al.,* (2008) reported thatreported the effective role of extracts obtained from different plants against *Fusarium sp.*

CONCLUSIONS

In the present study, it is concluded that the colony growth of the fungus was fast-growing, initially white later turned in yellowish, reddish, and golden brown. The small hyaline mostly single-celled microconidia were seen in long catenate chains arising from morphologically simple phialides.Therefore, the fungus was identified as *Fusarium moniliforme*. Whereas, the effect of Effect of plant extracts on root rot of Jatropha caused by *Fusarium moniliforme*, ten plant species belonging to different families observed the antifungal activity varied from one to another with different doses in an *in-vivo* experiment. Higher doses of Neem and Ginger extracts significantly decreased the disease incidence as compared to other plants and control. A variable effect of different fungicides and doses for disease incidence and plant growth parameters showed that at a higher dose of Aliette and Carbendazim significantly decreased the disease incidence, as compared to Metalyxl+Mancozeb, Thiomil, Clipper, and control.

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