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INORGANIC CONTROL OF PHOMOPSIS LEAF BLIGHT ON JATROPHA (*Jatropha curcas* L.)

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Inorganic control of Phomopsis leaf blight of *Jatropha curcas* was carried out to determine the Pathogenicity of the isolated organisms and establish the most appropriate fungicide(s) and rate(s) for the control of the diseases. The values of Phomopsis species spores count obtained and used for inoculation was 4921875spores/ml. It was revealed that, there was no significant difference with respect to the methods of inoculation. Similarly, there was no significant effect on number of days after inoculation and the fungal pathogen disease symptom appearance (incidence) on the leaves of Jatropha seedlings. Though not significant, 17 Days after Inoculation (DAI) recorded the highest value of fungal incidence (0.73%) which was closely followed by 11 DAI (0.53%). The least symptom on the leaves of seedlings was recorded at 9 and 13 DAI with 0.20% each. However, at 11DAI control had a higher incidence of 54.36% and severity of 34.57% followed by imidacloprid + metalaxyl-m + tebuconazole at normal dose with an incidence of 5.20%. At 13DAI, result shows control treatment to have an incidence of 66.83% and a severity of 36.67% which statistically differ from imidacloprid + metalaxyl-m + tebuconazole at normal rate with 11.09% incidence and a severity of 20.00%. Mancozeb was found to be very effective in the management of Phomopsis leaf blight.

KEY WORDS: - Phomopsis, Leaf blight, Jatropha and Fungicides

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INTRODUCTION

Fungi are actually microorganisms, however, and differ from plants such that they are now placed in their own kingdom. With the development of techniques needed for studying microorganisms, the discipline of mycology (Greek for *Mycos*=fungus+*-logy*=study) developed also, and now the fungi encompass organisms from many different groups. Indeed, not all fungi that cause plant disease are true fungi (Agrios, 2004). Most fungi are dispersed as spores through air currents, water, and animals (primarily insects). Fungi may also be spread in or on infected plant parts, movement of soil and on agricultural equipment. Fungi can destroy crops, and the economic consequences of this have been enormous throughout human history. Fungi reduce yield, destroy crops in the field and in storage and produce toxins poisonous to humans and animals. Blights, blasts, mildews, rusts, and smuts of grains (Alabi and Misari 2020).

The appearance of the disease is in circular spots on both leaf surfaces which finally widen and the leaf spoiled. Sometimes, it spreads further to the stem which may kill the plant, the old plant can also be infected, but the intensity of damage is lower than that found on seedling. The leaf colour usually changes initially to yellow and finally brown (Janick and Robert, 2008; Goto, 1992). The pathogen may attack the whole plant making it stunted and finally dies (Torres-caldaza *et al.,* 2011). Phomopsis, a genus of ascomyceta fungi cause dead-arm on *Jatropha curcas with* infections

usually beginning during early growth stages in spring; it affects leaves, fruits and shoot of plants. This disease causes the formation of lesions on shoots leaves and also fruit rot (Rodriguez and Redman, 2008). Seedling die-back and collar rot are also common in *Jatropha* nurseries, they can be severe when produced under irrigation in semi-arid environment (Anon. 2009). Other fungal diseases reported to infect *Jatropha* plant include; leaf spots, damping-off, root rot, rust and black mildew (Heller,1992 and Alexopoulus*et al*. 1996).

Fungicides are biocide chemical compounds or biological organisms used to kill or inhibit fungal spores (Margaret, 2004). Chemicals used to control oomycites, which are not fungi, are also referred to as fungicides since oomycites use the same mechanisms as fungi to infect plants (Schnabel and Jones, 2001). In contrast with most human medicines, most fungicides need to be applied before disease occurs or at the first appearance of symptoms to be effective, also, unlike many diseases of humans and animals, the damage caused by diseases on plants often does not go away, even if the pathogen is killed (Margaret, 2004). She further stressed that, fungicides can only protect new uninfected growth from a disease; also, few fungicides are effective against pathogens after they have infected a plant. This study was carried out to determine the Pathogenicity of the isolated organisms and establish the most appropriate fungicide(s) and rate(s) for the control of the diseases.

METHODOLOGY

Pathogenicity Trial in the Screen house

Raising of *Jatropha curcas* Seedlings

Certified seeds of *J. curcas* were obtained from Institute for Agricultural Research (IAR), ABU Zaria. The seeds were soaked in Sodium hypochlorite for five minutes then washed with sterile water; again, they were washed with 20 ml of alcohol and rinsed with sterile water to ensure safety against dust and other pathogens that may be present in the surface. Thirty-nine clay pots with diameter and depth of 25 cm and 24 cm respectively were washed, filled with heat sterilized soil and watered. Two seeds were sown in each pot and watered for 28 days under aseptic condition to prevent contamination. The seedlings were later thinned to one.

Inocula Preparation and Inoculation of Seedlings in Glasshouse for Pathogenicity Trial

The preserved pure cultures of the isolated pathogens were grown on PDAs in the laboratory until they sporulated. Ten (10) ml of sterilized distilled water was added to each Petri-dish and grown mycelia mat from the culture was harvested using a sterile scalpel. The mycelia were blended in an electric blender for five minutes, 200 ml of sterile distilled water was added in 500 ml conical flask and filtered using a double layer muslin cloth. Spores count was made using haemocytometer and compound microscope.

Spore concentration was calculated using the formula adopted by Marley (2013);

$$C = \frac{n}{256} \; 4 \times 10^6$$

Where:
C = number of conidia per millilitre
n = number of conidia counted in the chamber
256 = constant volume obtained from 16 x 16 square grids
4×10^6= constant

In vivo Evaluation of Fungicides in Glasshouse

The experiment was conducted in the glasshouse of the Department of Crop Protection, IAR, ABU, Zaria. Seeds of *J. curcas* meant for planting were obtained from the Department; they were soaked in Sodium hypochlorite for five minutes and then washed with sterile water to ensure safety against dust and other pathogens that may be present on the surface. One hundred and twenty plastic buckets were washed and filled with heat sterilized soil and watered for three days. Three seeds of *J. curcas* were planted in each bucket, after seedlings emerged; they were thinned to one per pot.

Thirty days after planting, fourteen days old cultures of *Phomopsis viticola* were used to inoculate the seedlings through soil, and leaves sprayed using hand atomizer. The most promising fungicides and rates obtained in the *in vitro* experiment were evaluated *in vivo* in the glasshouse and used to spray the seedlings three days after inoculation in a CRD. The experiments were run twice in three weeks.

Statistical Analysis

The data collected were subjected to analysis of variance (ANOVA) procedure using SAS (2012) software. Significant difference among the treatment means were separated using Duncan Multiple Range Test (DMRT).

RESULT

Determination of the Pathogenicity of the Isolated Organisms

Spores Count

The values of spores count obtained and used for inoculation was *Phomopsis* species 4921875 spores/ml. Effect of methods of inoculation on the incidence of fungal pathogens is shown in Table 1. It was revealed that, there was no significant difference with respect to the methods of inoculation (soil application, spray and smear) statistically. Similarly, results of fungal blight incidence on leaves of seedlings of *J. curcas* as influenced by number of days after inoculation is presented in table 2. It shows that, there was no significant effect on number of days after inoculation and the fungal pathogen disease symptom appearance (incidence) on the leaves of *Jatropha* seedlings. Though not significant, 17 Days After Inoculation (DAI) recorded the highest value of fungal incidence (0.73%) which was closely followed by 11 DAI (0.53%). The least fungal pathogen symptom on the leaves of seedlings was recorded at 9 and 13 DAI with 0.20% each. **(Plate 1)**

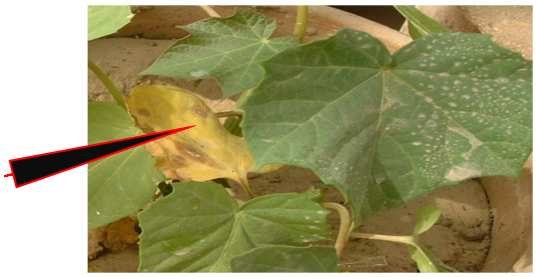

Plate 1: Phomopsis Leaf Light on Seedling of *Jatropha curcas*

Table 1. Effects of Method of Inoculation on Incidence of Phomopsis Blight on the Leaves of *Jatropha curcas Seedling*

SNO.	Inoculation Methods	Incidence (%)
1.	Soil	0.47±0.90
2.	Spray	0.37±0.62h
3.	Smear	0.33±0.71
	Significance	**Ns**

Table 2. Results on the Incidence of Phomopsis Blight on the Leaves of *Jatropha curcas* Seedlings as Influenced by Number of Days after Inoculation (DAI)

Days After Inoculation (DAI)	Incidence (%)
7	0.27±0.60
9	0.20±0.41
11	0.53±0.92
13	0.20±0.41
15	0.40±0.83
17	0.73±1.03
Significance	**Ns**

Evaluation of Fungicides on Leaf Blight of *Jatropha curcas* Caused by *Phomopsis viticola*

Result on the *in vivo* evaluation of fungicides on leaf blight of *J. curcas* caused by *P. viticola* is presented in Table 3 with significant difference among the treatments. At 7DAI, control treatment had an incidence and severity values of 17.72% and 20.00% respectively, while other treatments did not show any disease symptom, also at 9DAI similar trend was observed with control treatment having an incidence of 33.95% and severity of 5.35%, however, at 11DAI control

had a higher incidence of 54.36% and severity of 34.57% followed by imidacloprid + metalaxyl-m + tebuconazole at normal dose with an incidence of 5.20%. At 13DAI, result shows control treatment to have an incidence of 66.83% and a severity of 36.67% which statistically differ from imidacloprid + metalaxyl-m + tebuconazole at normal rate with 11.09% incidence and a severity of 20.00%. Other treatments had no visual symptom of leaf blight disease. At 15, 17 and 19DAI, control treatment maintained high incidence and severity followed by imidacloprid + metalaxyl-m + tebuconazole at normal concentration, with the highest incidence (81.56%) and severity of 68.27% at 21DAI followed by imidacloprid + metalaxyl-m + tebuconazole at normal rate with 16.39% and 20.29% as an incidence and severity values respectively while other treatments had zero values.

Table 3. In vivo Evaluation of Fungicides on Leaf Blight Caused by *Phomopsis viticola* (%)

Fungicides	Days after inoculation							
	7	9	11	13	15	17	19	21
	Incidence	Incidence	Incidence	Incidence	Incidence	Incidence	Incidence	Incidence
Dress force 2	0.00±0.00b	0.00±0.00b	5.20±5.69b	11.09±0.66b	12.09±0.64b	13.52±1.06b	15.06±1.68b	16.39±1.16b
Funguforce 2	0.00±0.00b	0.00±0.00b	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c
Funguforce 3	0.00±0.00b	0.00±0.00b	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c
Z-force 2	0.00±0.00b	0.00±0.00b	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c
Control	17.72±0.54a	33.95±5.35a	54.36±3.12a	66.83±3.29a	72.42±2.68a	76.68±2.05a	78.71±2.35a	81.56±1.27a

Fungicides	Days after inoculation							
	7	9	11	13	15	17	19	21
	Severity	Severity	Severity	Severity	Severity	Severity	Severity	Severity
Dress force 2	0.00±0.00	0.00±0.00	0.00±0.00b	20.00±0.00b	20.00±0.00b	20.28±0.02b	20.29±0.00b	20.29±0.02b
Funguforce 2	0.00±0.00	0.00±0.00	0.00±0.00b	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c
Fungu force 3	0.00±0.00	0.00±0.00	0.00±0.00b	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c
Z-force 2	0.00±0.00	0.00±0.00	0.00±0.00b	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c
Control	20.00±0.00	20.00±0.00	34.57±0.42a	36.67±2.16a	48.74±0.77a	53.64±1.97a	61.08±3.89a	68.27±0.78a

Means followed by the same letter(s) do not differ significantly according to Duncan Multiple Range Test (DMRT) at 5% level of significance.

DISCUSSION

In vitro and In vivo Evaluation of Fungicides

Mancozeb a contact fungicide applied alone had a remarkable impact on Phomopsis leaf blights at normal rate. Mostert et al. (2000) stated that, Mancozeb was comparable to both Kresoxym- methyl and azoxystrobin regarding its ability to inhibit mycelia growth of P. *viticola*. It inhibits spore germination. Generally, fungicides kill fungi by damaging their cell membrane, inactivating critical enzymes or proteins, or by interfering with key processes such as energy production or respiration (Hutson and Miyamoto, 1999). Others impact specific metabolic pathways such as the production of sterols or chitin.Lewin (2009) mentioned that many pathogens through simple mutation may develop resistance to some of the commonly used systemic fungicides within a few years of introduction. To avoid development of such resistant strains of the pathogens, it is always better to add one broad-spectrum contact fungicides along with the systemic fungicides at the time of application.

CONCLUTION

The use of a combined systemic and contact fungicide Mancozeb + Carbendazim and Imidlacloprid + metalaxyl-m + tebuconazole (a combination of systemic fungicides) applied at manufacturers recommended rate for the control of foliar blight caused by Phomopsis viticola are welcome development and prove to be effective, but Mancozeb singly was found to be very effective in the management of Phomopsis leaf blight.

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