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IN VIVO EVALUATION OF FUNGICIDES ON FUSARIUM EQUISETI FOLIAR BLIGHT OF JATROPHA CURCAS L.

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ABSTRACT

Three fungicides; Mancozeb, Mancozeb+ Carbendazim and Imidacloprid + metalaxyl-m + tebuconazole were evaluated *in vivo* at three levels (manufacturers recommended rate, half the recommended rate and one and half the recommended rate) on an isolate of *Fusarium equiseti* responsible for leaf blight on *Jatropha curcas* plant in a Complete Randomized Design. All the fungicides affected the mycelia growth of *Fusarium equiseti* at different levels. However, Mancozeb + carbendazim on one hand and imidlacloprid + metalaxyl-m + tebuconazole on the other hand proved to be the most effective on *Fusarium equiseti* when applied at manufacturers recommended rate, while mancozeb when used alone was only effective on the fungal pathogen at one and half recommended concentration.

Keywords: In vivo, fungicides, Fusarium equiseti and mycelial growth

INTRODUCTION

Jatropha curcas is a species of flowering plant in the spurge family, Euphorbiaceous, that is native to the American tropics, most likely Mexico and Central America [1]. Common names include Barbados nut, purging nut, physic nut, or JCL (abbreviation of *Jatropha curcas* Linnaeus) [2].

J. curcas is a poisonous, semi-evergreen shrub or small tree, reaching a height of 6 m (20 ft), it is resistant to a high degree of aridity, allowing it to be grown in deserts [1]. The seeds contain 27-40% oil (average: 34.4%) that can be processed to produce a high-quality biodiesel fuel, usable in a standard diesel engine [3]. When jatropha seeds are crushed, the resulting jatropha oil can be processed to produce a high-quality bio fuel or biodiesel tested successfully as fuel for simple

diesel engine that can be used in a standard diesel car or further processed into jet fuel [4]. Reference [5] added that, oil can be combusted as fuel without being refined, it burns with clear smoke-free flame, while the residue (press cake) can also be used as biomass feedstock to power electricity plants, used as fertilizer (it contains nitrogen, phosphorus and potassium), or as animal fodder, also cake can be used as feed in digesters and gasifiers to produce biogas.

Leaf blight, a disease caused by fungal pathogens results in the destruction of leaf tissue of which photosynthesis is reduced, this affects the growth and development of the Jatropha plant and subsequently its potentials, other diseases like root rot, caused by *Fusarium moniliforme*, causes wilt and death of Jatropha in waterlogged condition, and diseases like leaf spot, rusts, collar rot can also damage and kill *Jatropha curcas*. Insect pests affecting *Jatropha curcas* include army worms, aphids, mealy bugs, citrus root weevil etc. [6]. However, diseases and pests vary by climate and location depending on the growing area.

Fungicides are important tools for management of plant diseases caused by fungal and oomycete pathogens. However, fungicide usages need to be carefully planned with a good understanding of plant disease epidemics, their components (host, environment and pathogens), fungicide mode of action (biochemical, biological, physical), risk of resistance development and host physiology, among other aspects [7]. With the increasing demand for Jatropha as a source of biofuel in the world market [8], and several national governments' emphasison domestic sourcing of industrial raw materials, Jatropha has recently assumed a priority status for research and development [6].

The study aimed at determining the most appropriate fungicide(s) and rate(s) for the control of foliar blight disease of jatropha curcus caused by *Fusarium equiseti*.

MATERIALS AND METHOD

Sampling Technique

Based on the list of accessions obtained from Institute for Agricultural Research (IAR) Zaria, a Multi-stage purposive sampling was employed to select village locations with high population of *Jatropha curcas* in some north- west states of Nigeria. These were Tsaki, Kajiji and Barkeji in Kware, Shagari and Tambuwal Local Government Areas respectively from Sokoto State.

Isolation and identification

Diseased *J. curcas* leaves collected from the study area were sorted based on symptoms and taken to the pathology laboratory (Department of Crop Protection, I.A.R., Zaria) for isolation. The infected leaves were washed with distilled water, cut into pieces and sterilized for five minutes using 0.5% sodium hypochlorite and rinsed thrice with sterile distilled water and blotted

dry with a sterile filter paper (Rizvi and Yang, 1996). The pieces were then placed onto a 90 mm (diameter) petridishes containing freshly prepared Potato Dextrose Ager with streptomycin (PDAs) and labelled. The plates were incubated at 27^{o}C and observed daily [9]. Fungal mycelia of the isolated organisms were sub-cultured on fresh PDAs to obtain pure cultures. The cultural characteristics of the isolates were noted and detailed microscopic characteristics (morphological) were observed. The fungi were identified using Identification Manual of [10], further identification to species level was made by CAB International (United Kingdom). The pure cultures of the isolated fungal pathogens were preserved in McCartney bottles containing PDAs in a slanting position for inoculation.

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.

Inoculation

The preserved pure cultures of the isolated pathogens were grown on PDAs in the laboratory until they sporulated. 10 ml of sterilized distilled water was added to each petridish 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 [11];

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

Where:

C = number of conidia per milliliter

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 *Fusarium equiseti* was used to inoculate the seedlings through soil, and leaves sprayed using hand atomizer. Mancozeb, Mancozeb+ Carbendazim and Imidacloprid + metalaxyl-m + tebuconazole were used to spray the seedlings three days after inoculation in the glasshouse. The experiment was run twice in three weeks each.

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).

RESULTS

Result of *in vivo* evaluation of fungicides on leaf blight of *J. curcas* caused by *F. equiseti* is presented in Table 1 and 2. At 7DAI, control treatment recorded higher incidence of leaf blight (17.47%) while no disease symptom was observed in other treatments, similarly, high value of disease incidence (26.56%) was noticed in the control treatment and no leaf blight symptom was noticed in the mancozeb + carbendazim treatment at one and half concentration.

In both 7 and 9 DAI, severity values were not significantly different among the treatments. An incidence of 45.61% was recorded at 11 DAI in the control treatment and the least incidence of 10.29% was observed in the mancozeb + carbendazim treatment at one and half dose, however, higher severity (33.20%) was noticed in control treatment while it was statistically at par among other treatments. At 13DAI, control treatment maintained the highest incidence (61.42%) and the least (11.45%) in mancozeb + carbendazim at one and half rate, similar trend was also observed with respect to severity values. At 15 DAI control treatment still had higher incidence and severity of leaf blight (68.48% and 40.51%) respectively, while mancozeb + carbendazim and mancozeb at one and half concentration had the least incidence and severity. The same pattern was maintained at 17, 19 and 21 DAI.

Table 1: *In vivo* Evaluation of Fungicides on Leaf Blight (**Incidence)** Caused by *Fusarium equiseti* (%)

	Days after inoculation							
	7	9	11	13	15	17	19	21
Fungicides	Incidence	Incidence	Incidence	Incidence	Incidence	Incidence	Incidence	Incidence
Dress force 2	0.00±0.00b	10.58±0.13b	11.25±0.06b	12.17±0.04b	14.20±0.66b	17.46±0.03b	18.64±0.21c	19.41±0.06c
Fungu force 2	0.00±0.00b	10.50±0.40b	11.17±0.07b	12.49±0.05b	14.50±0.10b	17.44±0.14b	19.33±0.07b	21.34±0.17b
Fungu force 1	0.00±0.00b	10.28±0.12b	11.18±0.02b	12.25±0.10b	14.45±0.08b	17.73±0.08b	18.33±0.05d	19.64±0.13c
Fungu force 3	0.00±0.00b	0.00±0.00c	10.29±0.06c	11.45±0.03c	12.30±0.02c	14.45±0.02d	16.44±0.11f	17.41±0.13e
Z-force 3	0.00±0.00b	10.51±0.05b	11.20±0.55b	12.36±0.09b	14.42±0.06b	16.56±0.13c	17.31±0.06e	18.28±0.15d
Control	17.47±0.04a	26.56±0.75a	45.61±0.61a	61.42±0.95a	68.48±1.38a	78.70±0.95a	81.56±0.19a	83.44±1.03a

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

Table 2: *In vivo* Evaluation of Fungicides on Leaf Blight (**Severity**) Caused by *Fusarium equiseti* (%)

	Days after inoculation							
	7	9	11	13	15	17	19	21
Fungicides	Severity	Severity	Severity	Severity	Severity	Severity	Severity	Severity
Dress force 2	0.00±0.00	20.00±0.00	20.00±0.00b	20.15±0.03c	20.18±0.01c	20.55±0.54c	21.06±0.25b	21.06±0.25b
Fungu force 2	0.00±0.00	20.00±0.00	20.00±0.00b	20.16±0.02c	20.19±0.01c	20.76±0.55c	21.44±0.08b	21.44±0.08b
Fungu force 1	0.00±0.00	20.00±0.00	20.00±0.00b	20.21±0.03b	20.22±0.01b	21.54±0.07b	21.60±0.08b	21.61±0.07b
Fungu force 3	0.00±0.00	0.00±0.00	0.00±0.00b	20.00±0.00d	20.00±0.00d	20.00±0.00d	20.00±0.00c	20.00±0.00c
Z-force 3	0.00±0.00	20.00±0.00	20.00±0.00b	20.00±0.00d	20.00±0.00d	20.00±0.00d	20.00±0.00c	20.00±0.00c
Control	20.00±0.00	20.00±0.00	33.20±0.06a	33.25±0.07a	40.51±0.06a	45.89±0.24a	57.27±1.58a	76.18±1.41a

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

DISCUSSION

The fungicides evaluated in *vivo* showed variable response in inhibiting the colony growth of the fungal pathogens according to their nature and specificity when compared with the control

treatment. These results are in agreement with a number of earlier *in vivo* studies which have demonstrated that various fungicides may restrict or prevent growth of some fungal pathogens [12,13,14]. Mancozeb + Carbendazim (Fungu force) and imidacloprid + metalaxyl-m + tebuconazole (Dress force) showed a great inhibitory effect on mycelia growth in the leaf blight fungal pathogen when applied at the manufacturers recommended rate probably being combined systemic and contact fungicides and combined systemic fungicides respectively . High performance of tebuconazole was also reported by [15] that it had the greatest inhibitory effect in his experiment on *in vivo* inhibitory effect of selected fungicides on mycelial growth of ambrosia fungus causing 100% inhibition irrespective of the fungicide concentration or techniques employed. These results are in line with earlier research studies which reported very strong *in vitro* inhibition effects of tebuconazole-based fungicides on fungal mycelial growth of several *Fusarium* spp. For example, *F. avenaceum* [16;17], *F. culmorum* [16]. *F. graminearum* [17;18] and *F. verticillioides* [17] among others. Similarly, 100% mycelia growth inhibition by the fungicides tested agree with the findings of [5,19,20], being non systematic fungicides, they prevent infection largely by inhibition of spore germination and germ tube elongation [5], Mancozeb a contact fungicide applied alone had a remarkable impact on the Fusarium leaf blight at one and half recommended concentration. 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 [21]. Others impact specific metabolic pathways such as the production of sterols or chitin. For example, phenylamide fungicides bind to inhibit the function of RNA polymerase in oomycetes, while the benzimidazole fungicides inhibit the formation of betatubulin polymers used by cells during nuclear division [22].

Performance of mancozeb + carbendazim on *Fusarium equiseti* agrees with the results obtained in the study on the effect of conjoint carbendazim and mancozeb on *F.oxysporum* f.sp. *udum* after 72 h and 120 h of incubation which inhibited the growth of fungus at different concentrations of treatment as compared to control [23]. Also, it was revealed that significant performance by foliar spray of carbendazim (0.1%) and mancozeb (0.2%) was observed to have reduced leaf blight disease in mungbean plants under greenhouse conditions [24]. Reference [25] added that, use of Carbendazim in mixture with selective fungicides shows best results during *in vivo* and *in vitro* application on Pathogens infecting Fenugreek *(Trigonella foenum graecum. L),* by *Alternaria alternata (Fr.) Keissler,* causing root rot disease, nonetheless application of carbendazim-mancozeb fungicidal mixture, though useful in the control of fungal diseases, it should be use with caution. [26] stated that, use of higher rate of carbendazim-mancozeb mixture than the recommended does not offer any advantage in the control of target group, rather affects non-target ecologically important groups of microorganisms. Carbendazim used alone was not much effective on fungal pathogens at all rates used, this disagrees with the result obtained by [27]), that Carbendazim fungicide (50% SC) at four rates (0.1, 0.3, 1 and 3 mg/ml) evaluated on

in vitro radial growth of mycelia of *Fusarium oxysporum* f.sp. *Lycopersici* "strain F20" isolated from tomato (*Lycopersicon esculentum*) and *Colletotrichum capsici* "strain C226.3" isolated from chilli (*Capsicum annum* L) was observed at all the concentrations to have inhibited mycelial radial growth of the fungi, this could be attributed to difference in the species of pathogen and plant used.

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