Priming F1-resistant Tomato Hybrid (Lindo-F1) Seedlings with Copper-I-Oxide Metalaxyl Composite Fungicide before Infection Enhanced Resistance to Fusarium Wilt

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ABSTRACT

Vascular wilt disease caused by soilborne Fusarium oxysporum f.sp. lycopersici is a serious constraint to production of tomato in Southwestern Nigeria. F1-resistant tomato hybrid, Lindo-F1 seedlings were primed with Copper-I-Oxide Metalaxyl (CM) fungicide containing 65% Copper-I-Oxide in 12% Metalaxyl as a wettable powder (WP). The CM was suspended in Reverse Osmosis (RO) water and applied at four different concentrations (25, 42.5, 62.5, 87.5 mg Kg⁻¹) to the soil in the nursery, and the seedlings were assigned names (B-, C, D- and E-tomato plant) respectively, according to the concentration of CM treatment. The control (A-tomato plant) was primed with RO water. The aim was to elicit the plant's innate defences before pathogen challenge, in addition to the direct fungitoxicity of CM. Conidia suspension of three virulent, indigenous isolates of F. oxysporum f.sp lycopersici (Sensu lato) AWO-1, ERIO-1 and IGEDE-1 were tested against the water-primed- and CM-primed Lindo-F1 seedlings. Moreover, effects of the CM on crop...
performance indices, disease incidence and severity were evaluated for the genetically resistant tomato alone (water-primed) and the tomato seedlings primed with CM. The CM concentrations significantly affected the performance of the seedlings in the nursery, F (8, 75) = 9.358, P = 0.001, with a critical concentration (42.5 mg Kg⁻¹ soil), that adversely affected plants’ vigour. The rates of growth and development of the plants in the field in relation to the carry-over effects of CM-priming in the nursery was significant, F (6, 432) = 7.302, P = 0.001. The rates of flowering and fruiting in relation to the concentrations of CM treatments and the infecting strain of *F. oxysporum* also varied significantly, F(12, 972) = 5.796, P = 0.001. The severity of disease was significantly different among the treated and the control plants, F (18,576) = 2.143, P = 0.004. The C-Tomato plants produced the highest number of healthy and matured fruits.

**Keywords:** *Fusarium oxysporum*; tomato; vascular wilt disease; pathogens.

1. **INTRODUCTION**

Vascular wilt disease caused by soilborne *Fusarium oxysporum* f.sp. *lycopersici* is a serious constraint to the production of tomato in Southwestern Nigeria [1]. *Fusarium oxysporum* is a forma specialis that are pathogenic to different plant species and consist of different races within the specialised forms [2]. The severity of attack under different cropping systems and seasons vary, but attacks are severer in the rainy season when up to 100% loss of crops could occur.

Management of vascular wilt disease is responsible for a significant share of chemical fungicide use in tomato cropping systems in Nigeria. Successful control by the use of fungicides in the management of the disease may depend on the stage in the disease cycle at which treatment was applied [3]. *Fusarium oxysporum* attacks the transport system; thus the application of fungicides to tomato plants after infection and development of visible symptoms often fail to mitigate wilt. Chemical resistant strains of *F. oxysporum* have been characterised [4] and are often associated with environments where fungicides were used extensively. Environmental toxicity and presence of unacceptable pesticide residues in produce are also problems associated with repeated use of chemical fungicides, especially as foliar sprays.

*Fusarium oxysporum* is capable of growing as a saprophyte on plant debris in the absence of a susceptible host, and the macroconidia persist in the soil for many years [5, 6]. The bioecology of the pathogen, therefore, is a serious challenge to the use of crop rotation in the management of fusarium wilt disease. The existence of different races of *F. oxysporum* f.sp. *lycopersici* is also a serious hurdle in selecting a specific resistant hybrid for cultivation in *F. oxysporum* endemic area [2]. Plant defence stimulation based on non-toxic chemical substances, known as abiotic elicitors are capable of replacing traditional chemical fungicides. Elicitors are not toxic to pathogens, they are rather recognised by plant membrane receptors and induce plant's natural defence mechanism by stimulating innate immunity [7]. Elicitor-mediated defence against the pathogen is non-specific and are potentially capable of protecting crops against multiple pathogen challenges [8]. Examples of plant protection products that are strictly reliant on stimulation of innate defences to mitigate pathogen challenge include Laminarin (algae extract) [9] and Benzothiadiazole [10]. Other elicitors that are under evaluation are the chitooligosaccharides and oligogalacturonides [11]. Generally, elicitors are safe, and they have been associated with increased levels of phytoalexins in plants, with a positive effect on human health [12].

Elicitation of innate defences in plants is a physiological process that occurs over a period against biotic challenges, and the cycle is expected to vary with different plant species. An aggressive pathogen would likely overcome host’s defences if its speed of infection process supersedes the time required by the plant to potentiate its innate defences. At best, non-fungitoxic elicitors are likely to reduce the severity of disease rather than disease incidence [13, 14, 15]. Practically, this could be a potential setback to the exclusive use of elicitors for plant protection. We opined that fungitoxic substances that are elicitors would perform better, compared with the use of elicitors as stand-alone phytoprotectants.

There is a strong evidence that copper nanoparticles and metalaxyl, which are fungitoxic substances also act as phytoalexins elicitor in some crops [16,17,18]. Lazarovits & Ward [19] demonstrated increased phytoalexin production
in soybean sprayed with metalaxyl. Similarly, localised glyceolin accumulation following metalaxyl treatment in the control of phytophthora rot in soybean was reported [20]. There is no information on the effect of metalaxyl and copper-based substances on tomato concerning elicitation of innate defences, agronomic performance and resilience to fungal diseases in situ.

At present, delivery of chemical fungicides as a foliar spray in the management of vascular wilt disease of tomato have yielded limited positive outcomes in Southwestern Nigeria. Thus, there is interest to modify the mode of application of Copper-I-Oxide in Metalaxyl (CM) fungicide (Contact-Systemic composite fungicide) to Lindo-F1 tomato seedlings, such that the plant’s innate defences would be stimulated before pathogen challenge, in addition to the direct fungitoxicity of the compound. The CM was applied as soil-drench in the nursery to grow F1-resistant tomato hybrid (Lindo-F1) seedlings. Three virulent isolates of F. oxysporum f.sp lycopersici was tested against Lindo-F1 seedlings and CM-primed seedlings. Preliminary pathological studies [2] showed that Lindo-F1 tomato hybrid is variably susceptible to the three fungal isolates used in the current study. Moreover, crop performance indices: plant height, flowering and fruiting, as well as disease incidence and severity under field conditions, were evaluated for the genetically resistant tomato alone and the tomato seedlings primed with CM.

2. MATERIALS AND METHODS

2.1 Soil Preparation and Treatment with COPPER-I-Oxide + Metalaxyl

Four concentrations (1.0, 1.7, 2.5 and 3.5 g L⁻¹) of a composite fungicide containing 65% Copper-I-Oxide in 12% Metalaxyl as a wettable powder (WP) were prepared using Reverse Osmosis (RO) water. The fungicide has variable recommended standard application rates, depending on the crop and the target pathogen. Moist 20 kg subsamples of loam soil was autoclaved at 121°C, 15 psi for 30 minutes inside autoclave bags. The soil samples were left overnight to cool before they were poured into plastic nursery trays (L x B x H: 50 cm x 40 cm x 15 cm), which were previously disinfected by washing with 0.05% hypochlorite solution. The soil in each tray was drenched with 500 ml of the different concentrations of the prepared fungicide to obtain soil concentrations of 25, 42.5, 62.5, and 87.5 mg Kg⁻¹, while the soil for the control was drenched with water.

2.2 Effect of Copper-I-Oxide in Metalaxyl Fungicide on Seedling Growth

Thirty seeds of Lindo-F1 tomato hybrid were sown in the prepared nursery tray by drilling 10 seeds per row in three rows spaced 15 cm apart. The experimental set-up was a Randomized Complete Block Design (RCBD) with three replicate seed trays. The seedlings were irrigated with water as needed during the nursery period that lasted for three weeks. At two and three weeks after sowing, growth parameters; the height of seedlings (cm) were measured, number of leaves and the number of early branches were counted and recorded for 10 randomly selected seedlings from each tray. The seedlings were assigned names (A-, B-, C-, D- and E-tomato plant), according to the concentration of fungicide in the soil on which they were raised as shown in Table 1. A-Plant thus represents the non-fungicide-primed F1-Lindo tomato plants (genetic resistance quality alone).

2.3 Source of Fungal Isolates and Preparation of Inoculums

The F. oxysporum f.sp lycopersici (Sensu lato) were originally isolated from infected tomato plants in Nigeria and designated as AWO-1, ERIO-1 and IGEDE-1. Details of the source of the fungal pathogens, a method of isolation, growth relations and relative pathogenicity to F1-resistant tomato hybrid, Lindo-F1 were published elsewhere [2]. One centimetre agar plugs from cultures grown on standard Potato Dextrose Agar (PDA) (Sigma-Aldrich®) were transferred into freshly prepared plates. Conidia from fourteen days old culture of each isolate were harvested into RO water containing 0.02 % Tween 80% as a surfactant. Lindo-F1 tomato seeds were aseptically grown on sterile cotton wool in glass jars for 14 days, and seedling samples were inoculated with 0.5 ml of the prepared conidia of each isolate. The inoculated seedlings were incubated for 10 days at ambient temperature for infection to occur. Thereafter, the pathogen was isolated from the tomato seedlings and maintained on PDA at ambient temperature (25 ± 2°C). Conidia suspension was prepared from fourteen days old PDA culture of these recycled pathogens and standardised to 1 x 10⁶ conidia ml⁻¹ for use in subsequent pathogenicity assays. The re-pass of the Fusarium isolates through an infection cycle was
done to ensure the pathogens retain their virulence.

2.4 Transplanting, Pathogen Challenge and Evaluation of Crop Performance

Three weeks old fungicide-treated seedlings (B-, C-, D-tomato plants) and the control (A-tomato plants) were transplanted into prepared plots at standard spacing and staked. The E-tomato seedlings were stunted and not transplanted to the field for further evaluations. A two-centimetre deep hole was impressed around the root of each tomato stand, and 1 ml of the inoculum suspension of the different *F. oxysporum* isolates containing 1 x 10^6 conidia ml^-1 was delivered separately into the rhizosphere, in contact with the roots. The experiment was a RCBD with 20 replicates. At two and three weeks post-inoculation (plant age=5 and 6 weeks old respectively), the number of leaves and stem branches per plant were counted for randomly selected 12 plants. The number of flowers and fruits per plant was counted at 4 weeks post-inoculation (plant age=7 weeks old) and repeated weekly for an additional two weeks.

2.5 Assessment of Disease Incidence and Severity

Counting of withered branches per plant was done weekly at 2-5 weeks after inoculation. Disease severity (DS) was expressed as the number of withered branches (WB) per plant relative to a total number of branches per plant, as shown in Equation 2:

\[
DS = \frac{\text{Number of WB per plant}}{\text{Total number of branches per plant}} \times 100 \tag{1}
\]

All the tomato stands inoculated with ERIO-1 isolate, numbering twenty and the control plants were uprooted at the end of the experiment, seven weeks post inoculation (crop age=10 weeks). Thirty centimetre-portion of each plant was measured from the tip of the tap root to the stem and severed using garden secateurs. They were rinsed in water to remove sand and dissected longitudinally to reveal the presence of vascular streak (VS). The number of plants showing visible VS under a stereo-microscope was expressed as the percentage of the total number of plants examined, to evaluate disease incidence (DI) as shown in Equation 1. The plants inoculated with other *Fusarium* isolates were not considered in this evaluation.

\[
\text{DI} = \frac{\text{Number of plants with VS}}{\text{Total number of plants examined}} \times 100 \tag{2}
\]

2.6 Evaluation of Percentage Infected Fruits

Partially and fully riped fruits on A, B, C, D-tomato plants were harvested and examined for signs of Fusarium infection. They were thereafter sorted into diseased and uninfected samples under daylight. The percentage diseased fruits (DF) was calculated from the number of diseased fruits relative to the total number of fruits harvested, as shown in Equation 3.

\[
\text{DF} = \frac{\text{Number of diseased fruits}}{\text{Number of fruits harvested}} \times 100 \tag{3}
\]

2.7 Statistical Analysis

All the data collected were evaluated for compliance with the requirements of Parametric Statistical Procedure: equality of variance of the means was tested using the Leven's Test, and normality of distribution was assessed using Shapiro-Wilks procedure. Thereafter, the data were subjected to Analysis of Variance (ANOVA) procedure to compare means. Where there were statistically significant differences, post-hoc tests were conducted and means were separated using Tukey’s Honestly Significant Difference (HSD).

| Fungicide concentration in nursery soil (mg Kg^-1) | Name assigned to seedling (Plant groups) |
|-----------------------------------------------|------------------------------------------|
| 0                                            | A-tomato plant                           |
| 25                                           | B-tomato plant                           |
| 42.5                                         | C-tomato plant                           |
| 62.5                                         | D-tomato plant                           |
| 87.5                                         | E-tomato plant                           |
3. RESULTS

3.1 Effect of Fungicide Concentration in Soil on Seedling Growth and Development in Nursery

The mode of delivery of the CM composite fungicide to the soil was similar to methods of application of inorganic elicitors, to enable the tomato plant potentiate its innate defences before pathogen challenge. Statistical analysis of the effects of different concentrations of CM in soil on agronomic characteristics: seedling height, number of leaves and early branches, showed there were significant differences $F(8, 75) = 9.358$, $P=0.001$ (Table 2). The soil treatments generally reduced the performance of seedlings in the nursery. At 14 days in the nursery, the mean height, the mean number of leaves and the mean number of branches of the seedlings in control were 12.9 cm, 8.2 and 2.8 respectively and these values were significantly higher than recorded among plants growing on treated soil. The height of the seedlings growing on soils containing 42.5 and 62.5 mg Kg$^{-1}$ CM was not significantly different, being 7.3 cm and 6.8 cm respectively and the poorest seedling performance was recorded at 87.5 mg Kg$^{-1}$ treatment level. At CM concentrations of 25-87.5 mg Kg$^{-1}$, there were no statistically significant variabilities in the number of leaves and early branches (1-2 weeks in the nursery). However, at 21 days in the nursery, the mean number of branches on the tomato seedlings in control were 12.9 cm, 8.2 and 2.8 cm, 8.2 and 2.8 cm respectively and these values were significantly higher than recorded among plants growing on soils containing 42.5 and 62.5 mg Kg$^{-1}$ CM. The mode of delivery of the CM composite fungicide to the soil was similar to methods of application of inorganic elicitors, to enable the tomato plant potentiate its innate defences before pathogen challenge. Statistical analysis of the effects of different concentrations of CM in soil on agronomic characteristics: seedling height, number of leaves and early branches, showed there were significant differences $F(8, 75) = 9.358$, $P=0.001$ (Table 2). The soil treatments generally reduced the performance of seedlings in the nursery. At 14 days in the nursery, the mean height, the mean number of leaves and the mean number of branches of the seedlings in control were 12.9 cm, 8.2 and 2.8 respectively and these values were significantly higher than recorded among plants growing on treated soil. The height of the seedlings growing on soils containing 42.5 and 62.5 mg Kg$^{-1}$ CM was not significantly different, being 7.3 cm and 6.8 cm respectively and the poorest seedling performance was recorded at 87.5 mg Kg$^{-1}$ treatment level. At CM concentrations of 25-87.5 mg Kg$^{-1}$, there were no statistically significant variabilities in the number of leaves and early branches (1-2 weeks in the nursery). However, at 21 days in the nursery, the mean number of branches on the tomato seedlings in control were 12.9 cm, 8.2 and 2.8 cm, 8.2 and 2.8 cm respectively and these values were significantly higher than recorded among plants growing on soils containing 42.5 and 62.5 mg Kg$^{-1}$ CM. The soil concentrations of CM which had the least inhibitory effects on growth performance of the seedlings was between 25-42.5 mg Kg$^{-1}$. The seedlings grown on the soil which contained 87.5 mg Kg$^{-1}$ CM were stunted and not transplanted for further evaluations in the field.

3.2 Growth and Development of Tomato Plants after Pathogen Challenge

There were statistically significant variabilities in the rates of growth and development of the tomato plants in the field concerning CM-priming of seedlings in the nursery, and the *Fusarium* isolates, $F(6,432) = 7.302$, $P=0.001$. The performance of the treated plants at the early stage in the field (2-3 weeks after transplanting and infection) was related to the concentrations of CM to which the seedlings were subjected in the nursery soil. The inoculated strain of *Fusarium* also affected field performance of the tomato plants (Table 3). The foliage density and the rates of development of early branches were significantly the highest in control. The mean leaf count (MLC) on B$_2$, C$_-$ and D$_-$tomato plants inoculated with IGEDE-1 *Fusarium* isolate in the field were not significantly different being, 12, 14 and 24 leaves per plant respectively and the trend was consistent after three weeks. Whereas, the MLC on B$_2$, C$_-$ and D$_-$tomato plants inoculated with AWO-1 *Fusarium* isolate showed statistically significant variabilities in foliage density after three weeks: MLC= 58, 48 and 22 while the MLC in control was 91 leaves per plant. However, the field performance of D$_-$tomato plants was relatively poor and incomparable with other plant sets.

3.3 Flowering and Fruiting under Fusarium Wilt Pathogen Challenge

Table 4 shows the mean number of flowers and fruits on the tomato plants in the field after 4-6 weeks exposure to fusarium wilt pathogen. There

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**Table 2. Effects of concentrations of Copper-I-oxide in Metalaxyl in soil on growth and development of tomato hybrid, Lindo-F1 in the nursery**

| Growth Parameters | Soil concentrations of CM (mg Kg$^{-1}$) |
|-------------------|-----------------------------------------|
|                   | 0.0 | 25   | 42.5 | 62.5 | 87.5 | 14 days in nursery | 21 days in nursery |
| Plant Height (cm) | 12.9a | 8.4b | 7.3b,c | 6.8b,c | 5.4c |                   |                   |
| Number of leaves  | 8.2a | 5.8b | 5.4b | 5.6b | 5.1b |                   |                   |
| Number of early branches | 2.8a | 2.1b | 2.1b | 2.1b | 2.1b |                   |                   |

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**Table 4. Effects of concentrations of Copper-I-oxide in Metalaxyl in soil on growth and development of tomato hybrid, Lindo-F1 in the nursery**

| Growth Parameters | Soil concentrations of CM (mg Kg$^{-1}$) |
|-------------------|-----------------------------------------|
|                   | 0.0 | 25   | 42.5 | 62.5 | 87.5 | 14 days in nursery | 21 days in nursery |
| Plant Height (cm) | 17.5a | 11.0b | 11.0b | 8.3b,c | 6.9c |                   |                   |
| Number of leaves  | 9.9a | 7.2b | 7.2b | 6.7b,c | 5.1c |                   |                   |
| Number of early branches | 3.4a | 2.6a | 2.8a | 2.5a | 3.8a |                   |                   |
Table 3. Post-nursery growth and development of seedlings at two weeks and three weeks after inoculation with *F. oxysporum*

| Tomato plant groups | Growth characteristics | A-tomato plant | B-tomato plant | C-tomato plant | D-tomato plant | Fusarium isolates |
|---------------------|------------------------|----------------|----------------|----------------|----------------|------------------|
|                     | Number of leaves       | 49a            | 18b            | 20b            | 13b            | AWO-1            |
|                     | Number of branches     | 8a             | 4a,b           | 4b,c           | 3b             |                  |
| 2 weeks after       |                        |                |                |                |                |                  |
| inoculation with *F. oxysporum* conidia suspension (1ml/plant) | Number of leaves       | 26a            | 12b            | 12b            | 2c             |                  |
|                     | Number of branches     | 6a             | 4a,c           | 4c             | 1b             |                  |
|                     | Number of leaves       | 55a            | 12b            | 14b            | 24b            |                  |
|                     | Number of branches     | 10a            | 4b             | 4b             | 6b             |                  |
| 3 weeks after       |                        |                |                |                |                |                  |
| inoculation with *F. oxysporum* conidia suspension (1ml/plant) | Number of leaves       | 91a            | 58a,b          | 48b,c          | 22b            |                  |
|                     | Number of branches     | 11a            | 8a,b           | 6b,c           | 4b             |                  |
|                     | Number of leaves       | 81a            | 32b,c          | 39c            | 9b             |                  |
|                     | Number of branches     | 10a            | 6b             | 7b             | 2c             |                  |
|                     | Number of leaves       | 107a           | 30b            | 33b            | 55b            |                  |
|                     | Number of branches     | 13a            | 5b             | 5b             | 8a,b           |                  |
Table 4. Flowering and fruiting of tomato plants in the field at 4, 5 and 6 weeks after infection with *F. oxysporum*.

| Growth parameters | Tomato plant groups | Fusarium oxysporum inocula |
|-------------------|---------------------|---------------------------|
|                   | A-tomato plant      | B-tomato plant | C-tomato plant | D-tomato plant |
| 4 weeks after inoculation with *Fusarium oxysporum* conidia suspension (1ml/plant) | | | | |
| Number of flowers | 0.2a                | 0.3a           | 3.0a          | 2.7a          |
| Number of fruits  | 10.1a               | 0.0b           | 0.0b          | 0.0b          |
| Number of flowers | 0.0a                | 0.0a           | 3.1a          | 0.0a          |
| Number of fruits  | 6.5a                | 0.0b           | 0.0b          | 0.0b          |
| 5 weeks after inoculation with *Fusarium oxysporum* conidia suspension (1ml/plant) | | | | |
| Number of flowers | 0.3a                | 5.4a           | 39.5b         | 3.3a          |
| Number of fruits  | 29.7a               | 0.0b           | 2.6b          | 0.2b          |
| Number of flowers | 3.2a,c              | 38.2b          | 19.3a         | 0.0c          |
| Number of fruits  | 0.6a                | 20.5b          | 3.8a          | 2.5a          |
| 6 weeks after inoculation with *Fusarium oxysporum* conidia suspension (1ml/plant) | | | | |
| Number of flowers | 7.0a,c              | 36.1b          | 22.8a,b       | 0.5c          |
| Number of fruits  | 1.2a                | 35.5b          | 8.9a          | 4.8a          |
were statistically significant differences in the rates of flowering and fruiting concerning (a) the concentrations of fungicide applied to the soil on which the seedlings were raised at nursery stage and (b) the invading strain of \textit{F. oxysporum} \[F(12,972) =5.796, P=0.001\]. At four weeks, the mean flower count (MFLC) on plant samples inoculated with AWO-1 and ERIO-1 isolates were comparable, but the mean fruit count (MFRC) in the treated plants were significantly lower than the control. The control plants inoculated with IGEDE-1 isolate produced the highest number of fruits (MFRC=29.7), while the MFRC in the control plants inoculated with AWO-1 and ERIO-1 were 10.1 and 6.5 respectively. The CM treatments delayed the time to flowering and fruiting.

At 5 weeks post-inoculation with fusarium wilt pathogen, the MFLC and MFRC in the B-tomato plants were significantly the highest. In contrast, the MFLC and MFRC on D-tomato plants were the lowest. The B-tomato plants had the highest number of flowers (MFLC= 38.2, 30.9 and 40.7) and fruits (MFRC=35.5, 18.5 and 26.5) at six weeks, under AWO-1, ERIO-1 and IGEDE-1 \textit{Fusarium} attack respectively. In contrast, the MFRC in control were 3.2, 1.8 and 2.8 respectively. This trend was consistent and overall, the B-tomato plants produced the highest number of flowers and fruits.

### 3.4 Evaluation of Disease Incidence and Severity

Fig. 1 shows the number of the treated plants and the control inoculated with ERIO-1 \textit{F. oxysporum} isolate, which eventually developed visible streaks of vascular damage at ten weeks. Forty percent of the C-tomato plant samples had visible vascular tissue damage, and this was the lowest recorded disease incidence, while 80% of the control plants were affected. The disease incidence in the B and the D-tomato plants was comparable (50%). There were statistically significant variabilities in the severity of disease (mean count of withered branches) between the treated and the control plants, \[F (18,576) =2.143, P=0.004\]. The mean count of withered branches in the control plants increased significantly from 2-5 weeks after inoculation, indicating a consistent increase in disease progression, especially among the samples inoculated with AWO-1 and IGEDE-1 isolates of \textit{F. oxysporum} (Table 5). In contrast, there was no statistically significant increase in the mean count of withered branches between the control and the treated plants infected with ERIO-1 isolate from 2-5 weeks. The mean count of withered branches in the D-tomato plants infected by AWO-1 and IGEDE-1 at 2-5 weeks were 0.9, 0.9, 2.3, 4.3 and 0.2, 0.3, 0.3, 1.4 respectively. Comparably, the mean count of withered branches in the C-tomato plants infected by AWO-1 and IGEDE-1 at 2-5 weeks were 1.0, 0.5, 2.5, 4.3 and 0.2, 1.3, 1.7 and 3.7 respectively and these values were in most cases lower than the control.

### 3.5 Evaluation of Percentages of Fruits Infected by \textit{F. oxysporum}

The percentages of fruits showing signs of infection at harvest (Fig. 2) varied in relation to the infecting strains of \textit{F. oxysporum}, \[F(2,138)=8.368, P=0.001\] and treatments, \[F(3,138)=9.027, P=0.001\]. The percentage of infected fruits in control was significantly the highest, 15-45\% (Table 6). There were no infected fruits on the C-tomato plants under AWO-1 and ERIO-1 pathogen challenge. However, IGEDE-1 isolate damaged 17.9\% of the fruits. The percentages of fruits infected among the B- and the C- tomato plants were comparable.

### 4. DISCUSSION

The Copper-l-oxide in Metalaxyl composite fungicide applied as soil drench variably affected growth performance of seedlings in the nursery, with a detrimental critical concentration (42.5 mg Kg\(^{-1}\) soil), which suppressed seedlings growth, especially development of foliage, plant height and vigour. The fungicide contains 65 \% Copper-l-oxide in 12\% Metalaxyl as active ingredients of the formulation. At the tested concentration of 1g L\(^{-1}\) of water, 500 ml of the solution when applied to 20 kg soil sample, for example, delivered 0.65 g of Copper-l-oxide, equivalent to 0.02 mg/Kg of soil or 0.02 ppm. The study demonstrated that concentrations of the fungicide above 1.7 g L\(^{-1}\) affected seedling growth adversely. Plants respond to excess copper by depositing in the chloroplast, which could potentially alter physiological processes that affect growth and development [21-23]. Although the amount of copper in the tissues of the tomato seedlings was unverified, the presence of < 2.5 mg L\(^{-1}\) in soil was found toxic to brassicae [24] while concentrations in the range of 0.06-0.2 mg L\(^{-1}\) was reported as toxic to many other crops and implicated in poor seed germination, impaired development of foliage and stunted growth [25,
Similarly, priming of seed with Copper solutions to mitigate fungal infections variably affected seedlings performance in some studies [27]. It can be suggested that the relatively poor growth and development of the treated tomato seedlings, was in part caused by Copper toxicity. Thus, the mode of delivery of the Copper oxide-based antifungal was crucial to the performance of the seedlings.

Table 5. Mean number of withered branches (disease severity) among different plant groups after pathogen challenge

| Sampling for withered branches (Weeks after pathogen challenge) | A-tomato plant | B-tomato plant | C-tomato plant | D-tomato plant | Fusarium isolates |
|---------------------------------------------------------------|---------------|---------------|---------------|---------------|------------------|
| A-tomato plant                                               | 2.2a          | 3.0a,b        | 6.1b,c        | 9.5c          | AWO-1            |
| B-tomato plant                                               | 1.7a          | 1.2a          | 2.8a          | 5.3b          |                  |
| C-tomato plant                                               | 1.0a          | 0.5a          | 2.5a,b        | 4.3b          |                  |
| D-tomato plant                                               | 0.9a          | 0.9a          | 2.3a,b        | 4.3b          |                  |
| A-tomato plant                                               | 1.6a          | 0.9a          | 2.1a          | 5.8b          |                  |
| B-tomato plant                                               | 1.1a          | 1.0a          | 1.3a          | 3.5b          |                  |
| C-tomato plant                                               | 0.5a          | 0.8a          | 0.4a          | 0.8a          | ERIO-1           |
| D-tomato plant                                               | 0.9a          | 0.7a          | 1.8a          | 6.1b          |                  |
| A-tomato plant                                               | 0.1a          | 2.3b          | 1.1a,b        | 6.1c          |                  |
| B-tomato plant                                               | 0.2a          | 1.4a,b        | 0.8a          | 2.3b          |                  |
| C-tomato plant                                               | 0.2a          | 1.3a          | 1.7a          | 3.7b          |                  |
| D-tomato plant                                               | 0.2a          | 0.3a          | 0.3a          | 1.4b          |                  |

Values in the same row and sub-table not sharing the same subscript are significantly different at p<0.05 in the two-sided test of equality for column means. Tests are adjusted for all pairwise comparisons within a row of each innermost sub-table using the Bonferroni correction.

Table 6. Percentages of harvested fruits infected by *F. oxysporum* at harvest among plant groups

| *F. oxysporum* isolates | Concentrations of fungicide (g/l) | A-tomato plant | B-tomato plant | C-tomato plant | D-tomato plant |
|-------------------------|-----------------------------------|---------------|---------------|---------------|---------------|
| AWO-1                   |                                   | 15.26a        | .93b          | NIF           | 15.97a        |
| ERIO-1                  |                                   | 16.81a        | 3.43a         | NIF           | 6.88a         |
| IGEDE-1                 |                                   | 45.38a        | 9.26b         | 17.94a,b      | 12.97b        |

NIF=No infected fruit
At the early stage in the field (2-3 weeks after transplanting), the rates of development of foliage and growth of the fungicide-treated tomato plants were relatively lower than the control and the infecting pathogen also appeared to exert significant influence on plant performance. The time to flowering and fruiting was earlier in the untreated plants, however higher numbers of flowers and fruits were produced in the treated plants, especially the B-Plant and the C-plant. Also, a significant proportion of the flowers and the fruits in the control (A-Plant) aborted as the disease progressed (5 weeks after inoculation) and failed to mature, many of the fruits were infected and unsuitable for consumption. Eventually, the C-Tomato plants produced the highest number of healthy and matured fruits.

There is biochemical evidence that pre-application of inorganic chemicals to tomato plants before pathogen challenge cause increase in the concentration of phytoalexins (phenols), and other proteins associated with innate defences [28] and increased protection against disease [29]. Metalaxyl treatments have been shown to elicit phytoalexins production in soybean [19], but there is no data to compare the effects of metalaxyl treatments on growth performance of tomato.

Elicitors are detected in plants, which then switch on plant signalling system that result in defence reactions against invading pathogens. However, there are costs of fitness that plants incur in deploying their natural defences, measured in terms of vegetative developments, reproductive growth and yield. In earlier studies, it was suggested that a trade-off exists between effective disease control (elicitation of innate defences in plants), phytotoxic effects and plant productivity [13,30]. This may be responsible for the inability of the treated plants to compare favourably with the control regarding vegetative development at 2-3 weeks after transplant into the field and the delayed onset of flowering and fruiting.

The tomato species used in this study has a major pathogen-specific resistance gene (F1-resistance) thus, there was no record of completely wilted plants among the treated and the control, but there were variable signs of disease progression in the form of withered parts (branches), and this was used to assess the severity of infection. Disease occurrence was based on the number of plants which had a visible vascular streak at the end of the experiment when they were observed under microscope. The incidence of disease was lower and comparable among the treated plants, but disease incidence was significantly higher in control. Regarding the severity of disease, a consistent progress in disease was recorded in the control between 2-5 weeks after pathogen challenge, but a contrasting result was recorded among the treated plants. However, preliminary reports on the F. oxysporum isolates used in this study suggested that they are other races to which the tomato hybrid, Lindo-F1 was susceptible [2]. It is well known that plant defences are preconditioned by treatments that result in resistance or sometimes tolerance to pathogens and induced systemic resistance is ineffective once the pathogen has established. Thus success often depends on the appropriate
5. CONCLUSION

This study has demonstrated that elicitation of innate defences using Copper-I-Oxide Metalaxyl (CM) before pathogen challenge conferred some degrees of broad resistance and improved performance under multiple fusarium pathogen challenges. The treatment did not achieve complete control of the disease pathogen, it rather modulated severity of infection and the influence of CM on tomato was similar to non-fungitoxic elicitors. There are several studies on molecular aspects and regulation of induced resistance (IR), but little attention has been paid to field trials, where interactions of genotype and environment are capable of exerting influences on the expression of IR, agronomic traits and overall performance of tomato under a broad spectrum of Fusarium pathogen challenge. The results of this field trial showed the potentials of CM as inorganic elicitor in the management of fusarium wilt disease and the soil concentration of CM which had a negligible adverse effect on agronomic performances.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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