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Research Paper

Evaluation of heat treatment and bio-priming of cowpea seeds for the management of fusarium wilt disease in the screen house

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Abstract. Fusarium wilt is one of the most devastating diseases of cowpea. The pathogen, *Fusarium oxysporum* f.sp. *tracheiphilum* is soil and seed-borne. A non-chemical approach was designed for the management of the disease in the present study. Hot water (physical) at three temperatures, 40 50 and 60°C, and *Trichoderma viride* (biological) at 10^6 spores/ml were evaluated singly and synergistically in the laboratory. The duration of exposure to hot water was 5 min, after which seeds were allowed to cool followed by priming in sterile water (sole hot water treatment) or *T. viride* (synergistic physical and bio-control) medium in Petri-dishes for 72 h. The control consisted of seed treated with water at ambient temperature, while the standard check was seeds dressed with Mancozeb fungicide. Both were primed in a sterile water medium. Nine treatments were evaluated in all. Germinated seeds were transplanted into plastic pots containing soil infested with *F. oxysporum* at 10^6 spores/ml in the screen house. The experimental layout was completely randomized design and data collected were subjected to statistical analysis and mean separation. Results showed that seeds treated with 50°C hot water only had the highest germination percentage, 96.66%, and seedling vigour, 460.98. Disease incidence and severity values were least in seeds treated with synergistic hot water at 50°C and priming in *T. viride* medium. The highest number (77.33) and weight (13.41 g) of seeds were also recorded for the same treatment, while the least, 18 and 3.60 g were obtained from control. Integrated use of hot water (50°C) and bio-priming in *T. viride* medium can be recommended for use in the management of fusarium wilt disease of cowpea.

Keywords: Fusarium wilt, cowpea, hot water, *Trichoderma viride*, germination, vigour, yield.

INTRODUCTION

Cowpea [*Vigna unguiculate* (L.) Walp], is believed to have originated from Africa (Baudoin and Marchal, 1985; Pasquet, 2000; FAO, 2004). It is a member of the Fabaceae family and is cultivated mostly in the arid, semi-arid tropic and sub-tropical countries. This is due to its hardiness and tolerance to drought stress (Muoneke *et al*., 2012; Ajayi *et al*., 2018). The crop is important for its numerous agricultural, nutritional and health benefits (Owolade *et al*., 2006; Singh *et al*., 2006; Tariku, 2008). It is an important source of cheap protein for millions of poor Africans who cannot afford other sources like meat and egg that are considered to be more expensive.

Nigeria is a leading producer of cowpea in the world (FAO, 2014) but pests and diseases have remained a serious challenge threatening production, especially in the humid rain forest zone (Singh *et al*., 1990; Adegbite and Amusa, 2008). Significant annual yield losses have been reported (Muhammed and Sajo, 2018, Kusi *et al*., 2019). One of the most devastating diseases of cowpea in Nigeria is Fusarium wilt. A strain of the causative organism was identified as *F. oxysporum* f.sp. *tracheiphilum* Race 1 in 1980 (Armstrong and Armstrong, 1980). *F. oxysporum* is

characterized by a cotton-like appearance on growth medium. The colour may be white, brown, pink or violet, depending on the strain (Summerell *et al*., 2003). Microscopy reveals a characteristic curved or canoe-shaped macro and microconidia that occur singly and with oblong or ovoid shapes. The mycelium is typically hyaline and septate (Barnette and Hunter, 2006). *F. oxysporum* is soil-borne and can survive from one cropping season to another as resistant chlamydospores. The pathogen gains entrance into the tissue of the host plant through the root hairs. It then moves into the vascular tissue, where it obtains nutrients, and multiply rapidly. Conducting tissues are blocked after a given period of infection, preventing the transportation of water and nutrients to parts of the plants where they are required. This eventually leads to yellowing of leaves, followed by wilting, premature defoliation and death of plant (Tiwari *et al*., 2018). The incidence of the disease has been reported in many cowpeas growing regions of the world (Pottorf *et al*., 2012; Wu *et al*., 2015; Berger *et al*., 2016). Infection from *Fusarium oxysporum* on crops is so devastating that the pathogen has been ranked as 5th out of the top 10 pathogens of economic importance in the world (Van Kan Jal *et al*., 2012).

The most effective option available for the management of fusarium wilt of cowpea is the use of resistant varieties (Pottorf *et al*., 2012). Resistant cowpea varieties are however not always available in the rural tropics. Most peasant farmers in this region use seeds from the previous harvest, which may already be contaminated or infected with the pathogen, as planting material in the next cropping season. Benomyl, Carbendazim, Captan and Dithane have all been shown to produce varying degrees of inhibition on *Fusarium* spp. (Amini and Sidovich, 2010; Khare *et al*., 2016). Abnormal cell division in plants, destruction of useful and non-target soil microorganisms as well as toxicity and disruption of the normal reproductive cycle in humans are some of the harmful effects associated with the use of these chemicals (Dane and Dalgic, 2005; Fawole *et al*., 2009., Ge *et al*., 2018). Heat treatment has shown promise in the management of some plant diseases, especially those that are seed-borne (Gilbert *et al*., 2005) and that may be present as contaminants, while biological control has been used successfully to manage certain diseases of crops incited by *Fusarium* spp. (Khan *et al*., 2004). This study evaluated hot water (physical) and *Trichoderma viride* (biological) singly and synergistically, which is novel, for the management of fusarium wilt of cowpea.

The general objective of the study is to provide a management option for fusarium wilt of cowpea that involves minimal or non-usage of synthetic chemical, while the specific objectives were; i. to determine if hot water and *T. viride* as sole and synergistic treatment confer protection on cowpea seeds against *F. oxysporum* f.sp*. tracheiphilum*, the causal agent of Fusarium wilt. ii. to access the effect of treatments on cowpea growth and yield parameters.

MATERIALS AND METHODS

Preparation of artificial growth medium

Potato dextrose agar (PDA) was used as the growth medium and preparation was based on manufacturers recommendation. Prepared PDA was sterilized in a Surgifried autoclave, 2010 model, at 121°C for 15 min. Amendment, to prevent bacterial growth, was with lactic acid, while pour plating, 15 ml/ Petri-dish, was done when sterilized PDA cooled to 45°C.

Isolation and identification of *Fusarium oxysporum* f.sp. *tracheiphilum*

About 0.5 kg of cowpea seed was purchased from a local shop a few meters away from the south gate of The Federal University of Technology, Akure (FUTA). It was brought to the pathology laboratory of Crop, Soil and Pest Management department (CSP) and examined carefully for symptoms/signs of Fusarium wilt disease. Seeds suspected to be infected were selected, surface-sterilized in 0.2% Sodium Hypochlorite for 2 min, rinsed in four changes of sterile distilled water and inoculated on gelled PDA. Two seeds were inoculated per Petri-dish and incubated for 48 h. Sub-culturing was done thereafter. Isolates with morphological features synonymous with *Fusarium* spp. were selected for further study. Microscopy and pathogenicity test were carried out to ascertain the authenticity and virulence of the isolate, while samples were also taken to the pathology unit of International Institute of Tropical Agriculture (IITA) Ibadan for confirmation.

Isolation and identification of *Trichoderma viride*

Trichoderma viride was isolated from moist garden soil. A small quantity of the soil sample, about 5 g, was transported to the pathology laboratory of CSP Department in a sterile specimen bottle. A small portion of the sample, about 0.3 g was sprinkled on the surface of gelled PDA in 5 Petri-dishes and incubation at 28°C. Observation for fungal growth was at 24 h interval, while sub-culturing was done until a pure culture of *T. viride* was obtained and confirmed through it morphological characteristics on culture plates and microscopy, assisted by the description and illustration of Barnett and Hunter (2006).

Collection and sterilization of soil

Sandy loam soil was collected from the teaching and research farm (TARF) and transported to CSP screen house the same day. Sterilization of the soil was done using steam heat method. Sterile soil was dispensed,

Figure 1. Experimental layout of primed and germinated cowpea seedlings in Petri-dishes containing absorbent paper moistened with the different treatments evaluated.

after cooling, into 4 L plastic pots at the rate of 4 kg per pot. Each pot had seven small holes, about 3 mm in diameter, at the base to allow for easy drainage of excess water.

Infestation of sterilized soil with spore suspension of *F. oxysporum*

Spores were harvested from axenic cultures of *F. oxysporum* from 38 Petri-dishes. Thirty (30) milliliter of sterile water was used to harvest spores from each Petri-dish, making use of sterile spatula. Harvested spores, obtained after filtration with a thin layer of sterile cotton wool, were adjusted by the addition of sterile water as appropriate to 10^6 spores/ml, making use of a haemocytometer slide. Seventy (70) milliliter of the spore suspension was sprayed evenly on the surface of well moistened sterile soil in each plastic pot. Infested soils were kept in the screen house for 48 h, after which transplanting of primed cowpea seedlings was done.

Collection and treatment of test crop

The test crop was Ife-brown Tvu 3629 variety of cowpea. It was developed by and obtained from IITA Ibadan, Oyo State, Nigeria. The choice was informed by the popularity of and the preference most Nigerian has for the brown variety of cowpea. Its short life cycle and determinate growth pattern also made it a good research material. Sole hot water and *T. viride* treatment, as well as a combination of both, were evaluated. The control consisted of soil infested with *F. oxysporum* but untreated cowpea seeds, while the standard check consisted of cowpea seeds dressed with Mancozeb fungicide at the manufacturer's recommended rate. Hot water at three temperatures (40, 50 and 60°C) was evaluated. Ninety (90) cowpea seeds each were subjected separately to each temperature for 5 min in a covered thermos flask. The flask contained 200 ml of water at each of the specified temperatures. At the expiration of 5 minutes, seeds were removed and allowed to cool before the application of further treatments. Cooled seeds were primed in Petri-dishes containing sterile absorbent paper moisten with 20 ml sterile water (sole hot water treatment) or 10^6 spore suspension of *T. viride* (synergistic hot water and *T. viride* treatment). Each treatment was replicated three times at the rate of 30 seeds per Petri-dish. The control consisted of seeds moistened with water at ambient temperature for 5 minutes, while the standard check was made up of seeds treated with a solution of Mancozeb fungicide for 5 min. Both were primed in absorbent paper moistened with sterile distilled water. Priming lasted for 72 h for all treatments.

Twenty seedlings, out of the total that germinated in each Petri-dish, replicate, during priming (Figure 1), were transferred to infested soils in plastic pots at 48 h after infestation. Each seedling was submerged in infested soil and each treatment was replicated three times. The number of stands in each pot was reduced to two at one-week after emergence (Figure 2).

Experimental design and layout

The experiment was in two stages, laboratory and screen house. The layout was completely randomized design (CRD) in both the laboratory and the screen house. The 9 treatments evaluated are listed as follows:

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1. Hot water, 40°C, treatment for 5 min and priming in a sterile water medium for 72 h. HW40.
2. Hot water, 50°C, treatment for 5 min and priming in a sterile water medium for 72 h. HW50.
3. Hot water, 60°C, treatment for 5 min and priming in a sterile water medium for 72 h. HW60.
4. Hot water, 40°C, treatment for 5 min and priming in *T. viride* medium for 72 h. HW40T.

Figure 2. Plastic pots containing two cowpea stands per pot laid out in a completely randomized design (CRD) in the screen house.

5. Hot water, 50°C, treatment for 5 min and priming in *T. viride* medium for 72 h. HW50T.
6. Hot water, 60°C, treatment for 5 min and priming in *T. viride* medium for 72 h. HW60T.
7. Ambient temperature for 5 min and priming in *T. viride* medium for 72 h. T. ONLY.
8. Ambient temperature for 5 min and priming in a sterile water medium, CONTROL.
9. Seed dressing with Mancozeb for 5 min and priming in sterile water for 72 h. S. CHECK.

Data collection and statistical analysis

Data were collected on the following parameters:

i. Germination rate

The number of seeds with emerged radicle was counted daily for 3 days. The values obtained in each treatment were converted to percentage seed germination making use of a simple mathematical relationship

$$psg = \frac{gs}{nse} \times 100$$

Where,
psg = percentage seed germination
gs = germinated seedlings
nse = number of seeds evaluated

ii. Seedling length

The length of all germinated seedlings in each Petri-dish was measured with a ruler at 72 hours after priming commenced. The mean value was obtained and expressed in cm as the seedling length for each treatment.

iii. Seedling vigour

This was obtained using the modified method of Abdul Baki and Anderson (1973)

$$\mathrm{SV} = Seedling\ length \times \%\, Seed\ germination.$$

Where SV = Seedling vigour

iv. Seedling establishment

The number of seedlings that survived in *F. oxysporum* infested soil one week after transplanting were counted and expressed as percentage seedling establishment using the formula:

$$pse = \frac{nes}{nts} \text{ x } 100$$

Where,
pse = percentage seedling establishment
nes = number of established seedlings
nts = number of transplanted seedlings

v. Disease incidence

Data collection on disease incidence started from the 4th week after transplanting. The number of leaves showing symptoms of Fusarium wilt in each treatment were counted and expressed in percentage with the formula:

$$di = \frac{nl}{tnl} \times 100$$

Where,
di = disease incidence
nl = number of leaves showing symptoms of infection
tnl = total number of leaves on a plant

vi. Disease severity

Data on disease severity was collected once a week from the 4th weeks after transplanting. Each plant was examined carefully for symptoms of Fusarium wilt. Severity score was awarded based on he adopted severity scale of Pottorf *et al*. (2012). It consisted of a 0 to 5 rating scale as follows:

0 = Healthy plant showing no sign or symptom of the disease.

1 = Approximately 10% of plant shows symptoms of infection from *F. oxysporum.*
2 = Approximately 25% of plant shows symptoms of infection from *F. oxysporum.*
3 = Approximately 50% of plant shows symptoms of infection from *F. oxysporum.*
4 = Approximately 75% of plant shows symptoms of infection from *F. oxysporum.*
5 = Entire plant shows symptom of infection from *F. oxysporum*/death.

vii. Plant height

This was measured weekly, starting from the 4th week, with a meter rule. Measurement was from the surface of soil inside each pot to the shoot apex. The values obtained were recorded for each treatment.

viii. Number of leaves

The number of leaves for each treatment was obtained through visual count once in a week, starting from the 4th week after transplanting. Only completely opened leaves were counted, while those in bud stage or partially opened were ignored. The number obtained was recorded for each treatment.

ix. Yield quantity and quality

At maturity, the pods produce by each treatment was harvested and shelled manually. The total number of seeds produced by each treatment was counted and recorded. Also, the entire seeds produced by each treatment were weighed, while the value obtained was also recorded and expressed in grams.

All data collected were subjected to analysis of variance (ANOVA) with the aid of Minitab (version 17) software. Mean separation at 5% level of probability was achieved with the use of Tukey's test.

RESULTS

Effect of treatments on germination rate

Most treatments were statistically similar with regards to germination rate at all the periods, 24, 48 and 72 h after sowing, that data were collected. The highest percentage germination at 24 h after sowing was 43.33%. It was obtained from HW40. The least value was 25.00%, from HW40T (Table 1). Germination rates of 90.00% and 96.66% were recorded at 48 and 72 h after priming commenced (APC) from HW50. These were the highest values for both periods. T. ONLY and HW50T recorded the least germination percentages of 70.00% and 76.66% respectively at 48 and 72 h APC (Table 1). It is worthy of note that the treatments did not seem to have any serious adverse effect on overall germination, as all recorded germination percentage of 70% and above at 72 h APC (Table 1).

Effect of treatments on seedling length

A general observation was that seed priming in *T. viride* medium, after hot water treatment, resulted in shorter seedlings compared to seeds that were primed in sterile water medium after hot water treatment (Figure 3). Seedlings length differed significantly among some of the treatments, and the highest value, 4.79 cm, was obtained from HW50, while the least, 3.10 cm was from HW40T (Figure 4).

Effect of treatments on seedling vigour

Seedling vigour in most treatments were similar statistically. With a value of 460.98, HW50 had the best vigour. This value was significantly higher than the other treatment (Figure 5). HW60 was next with 414.38 seedling vigour, while HW50T had the least value at 290.70. HW40, T. ONLY, CONTROL and S. CHECK were all similar statistically. Interestingly, sole hot water treatment of seeds at the three selected temperatures produce seeds with more vigour than those subjected to *T. viride* priming after hot water treatment.

Effect of treatments on seedling establishment

Seedling establishment was statistically similar among most treatments. At one week after transplanting (WAT), 95.00% of HW50T seeds survived. This was the highest value and it differed significantly from all other treatments (Figure 6). HW60T had the least value, 31.66%, while HW60 had the second-lowest value at 60.00%. The remaining 6 treatments were all not significantly different. Each had seedling establishment values above 70% (Figure 6).

Effect of treatments on disease incidence

No definite pattern of disease incidence was recorded for the treatment across the weeks for which data were collected (Table 2). While disease incidence decreased with increasing age in HW40T, the reverse was the case in HW40, HW50T and HW60T. An important finding, however, was that disease incidence was consistently and significantly lowest in HW50T with the values of 3.48, 6.64 and 7.63% at 5, 6 and 7 WAT, respectively. The

Table 1. Effect of treatments on cowpea seed germination (%).

Treatments	Germination / Hours after sowing		
	24	48	72
HW40	43.33^{a}	83.33^{ab}	91.66^{ab}
HW40T	25.00^{c}	80.00^{abc}	95.00^{a}
HW50	36.66^{bc}	90.00^{a}	96.66^{a}
HW50T	40.00^{ab}	71.66^{bc}	76.66^{c}
HW60	41.66^{ab}	81.66^{abc}	90.00^{ab}
HW60T	36.66^{abc}	75.00^{bc}	86.66^{abc}
T. ONLY	28.33^{bc}	70.00^{c}	81.66^{bc}
CONTROL	30.00^{abc}	73.33^{bc}	80.00^{bc}
S. CHECK	33.33^{abc}	78.33^{abc}	88.33^{abc}

Note. Means on the same column followed by the same subscript are not significantly different (P< 0.05) based on Tukey's test.
LEGEND: 1. HW40. Hot water (40°C) treatment for 5 min and priming in sterile water medium for 72 h.
2. HW50. Hot water (50°C) treatment 5 min and priming in sterile water medium for 72 h.
3. HW60. Hot water (60°C) treatment for 5 min and priming in sterile water medium for 72 h.
4. HW40T. Hot water (40°C) treatment for 5 min and priming in *T. viride* medium for 72 h.
5. HW50T. Hot water (50°C) treatment for 5 min and priming in *T. viride* medium for 72 h.
6. HW60T. Hot water (60°C) treatment for 5 min and priming in *T. viride* medium for 72 h.
7. T. ONLY. Water at ambient temperature for 5 min and priming in *T. viride* medium for 72 h.
8. CONTROL. Water at ambient temperature for 5 min and priming in sterile water medium for 72 h.
9. S. CHECK. Seed dressing with Mancozeb solution for 5 min priming in sterile water for 72 h.

Figure 3. Seedling lengths of cowpea: (a) HW40, (b) HW40T, (c) HW50, (d) HW50T, (e) HW60, (f) HW60T.

CONTROL had significantly highest values of 19.58, 19.19 and 31.44% for the same period (Table 2).

Effect of treatments on disease severity

The highest disease severity values, 4.50, 4.33 and 4.33, at the 5th, 6th and 7th WAT respectively, were obtained from CONTROL (Table 3). These values were significantly different from the other treatments. Significantly lowest disease severity values of 1.33, 1.66 and 1.33 were recorded for HW50T at the same period of 5, 6 and 7 WAT. Disease severity value was also high in HW40T (3.33) at 5 and 6 WAT respectively. The values

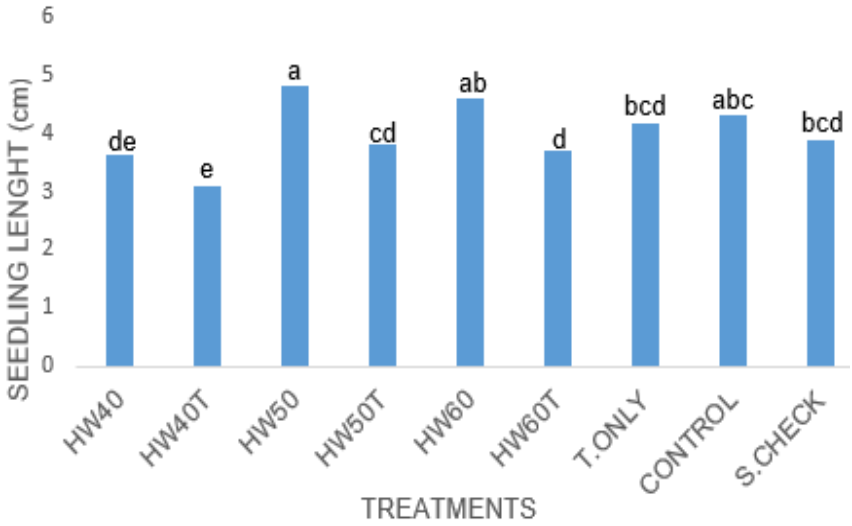

Figure 4. Effect of treatments on seedling length of cowpea (cm).
LEGEND: 1. HW40. Hot water (40°C) treatment for 5 min and priming in sterile water medium for 72 h.
2. HW50. Hot water (50°C) treatment 5 min and priming in sterile water medium for 72 h.
3. HW60. Hot water (60°C) treatment for 5 min and priming in sterile water medium for 72 h.
4. HW40T. Hot water (40°C) treatment for 5 min and priming in *T. viride* medium for 72 h.
5. HW50T. Hot water (50°C) treatment for 5 min and priming in *T. viride* medium for 72 h.
6. HW60T. Hot water (60°C) treatment for 5 min and priming in *T. viride* medium for 72 h.
7. T. ONLY. Water at ambient temperature for 5 min and priming in *T. viride* medium for 72 h.
8. CONTROL. Water at ambient temperature for 5 min and priming in sterile water medium for 72 h.
9. S. CHECK. Seed dressing with Mancozeb solution for 5 min priming in sterile water for 72 h.

were not significantly different from CONTROL. Disease severity was generally higher in some of the treatments at 6 WAT (Table 3).

Effect of treatments on plant height

Data on plant height was collected from the 4th to the 8th WAT. Plant height increased steadily in all treatments, but the rate of increase differed from one treatment to another. The highest values, 23.61 cm, 30.20 cm and 38.41 cm at 4, 6 and 8 WAT respectively were obtained from HW50T. The values at 4 and 8 WAT differed significantly from all other treatments, but that of 6 WAT was statistically similar to T. ONLY (Table 4). HW60T had the least plant height values at 4 and 6 WAT, 18.58 and 21.75 cm respectively. The values were however not significantly different from CONTROL in the same period. At 8 WAT, CONTROL produced significantly shortest plants with 24.80 cm height (Table 4).

Effect of treatments on leaf production

The number of leaves increased steadily from the 4th to 6th WAT but declined in almost all the treatments from 6 to 8 WAT, except HW50T, T. ONLY and S. CHECK (Table 5). HW50T had significantly highest number of leaves at 6 and 8 WAT. The treatment also had the highest number at 4 WAT but was not significantly different from HW50. CONTROL had 8.00, 14.00 and 12.66 numbers of leaves at 4, 6 and 8 WAT respectively. These values were consistently the least and were significantly lower than all other treatments (Table 5).

Effect of treatments on yield

The quantity and quality of seeds were affected by the different treatments. The total number of seeds produced was statistically similar in most of the treatments (Figure 7). HW50T produced 77.33 seeds that weighed 13.41 g. These values were the highest and each differed

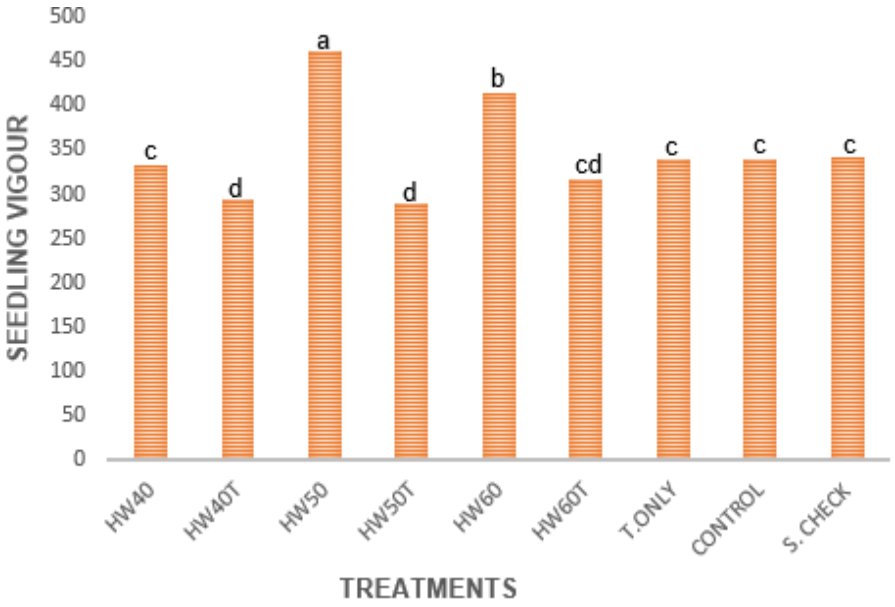

Figure 5. Effect of treatments on seedling vigour of cowpea.
LEGEND: 1. HW40. Hot water (40°C) treatment for 5 min and priming in sterile water medium for 72 h.
2. HW50. Hot water (50°C) treatment 5 min and priming in sterile water medium for 72 h.
3. HW60. Hot water (60°C) treatment for 5 min and priming in sterile water medium for 72 h.
4. HW40T. Hot water (40°C) treatment for 5 min and priming in *T. viride* medium for 72 h.
5. HW50T. Hot water (50°C) treatment for 5 min and priming in *T. viride* medium for 72 h.
6. HW60T. Hot water (60°C) treatment for 5 min and priming in *T. viride* medium for 72 h.
7. T. ONLY. Water at ambient temperature for 5 min and priming in *T. viride* medium for 72 h.
8. CONTROL. Water at ambient temperature for 5 min and priming in sterile water medium for 72 h.
9. S. CHECK. Seed dressing with Mancozeb solution for 5 min priming in sterile water for 72 h.

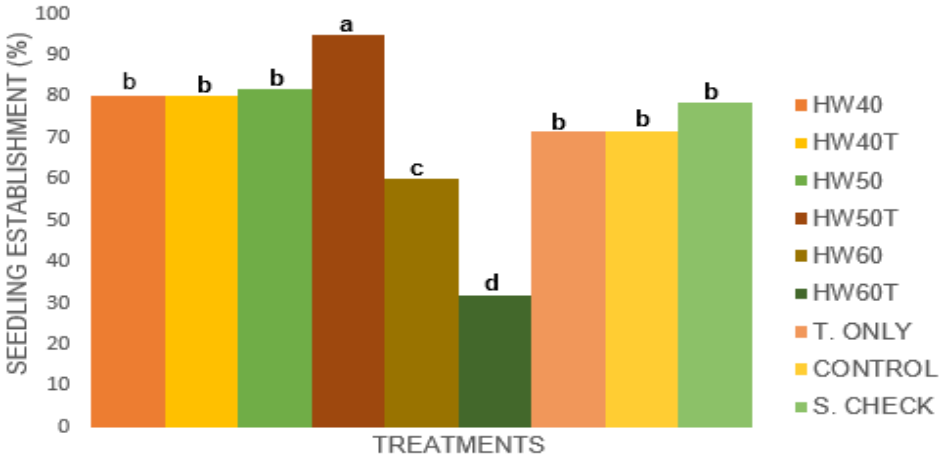

Figure 6. Effect of treatments on cowpea seedling establishment.
LEGEND: 1. HW40. Hot water (40°C) treatment for 5 min and priming in sterile water medium for 72 h.
2. HW50. Hot water (50°C) treatment 5 min and priming in sterile water medium for 72 h.
3. HW60. Hot water (60°C) treatment for 5 min and priming in sterile water medium for 72 h.
4. HW40T. Hot water (40°C) treatment for 5 min and priming in *T. viride* medium for 72 h.
5. HW50T. Hot water (50°C) treatment for 5 min and priming in *T. viride* medium for 72 h.
6. HW60T. Hot water (60°C) treatment for 5 min and priming in *T. viride* medium for 72 h.
7. T. ONLY. Water at ambient temperature for 5 min and priming in *T. viride* medium for 72 h.
8. CONTROL. Water at ambient temperature for 5 min and priming in sterile water medium for 72 h.
9. S. CHECK. Seed dressing with Mancozeb solution for 5 min priming in sterile water for 72 h.

Table 2. Effect of treatments on the incidence of fusarium wilt on cowpea plant (%).

Treatments	Disease incidence/ Weeks after transplanting		
	5	6	7
HW40	13.77^{cd}	15.49^{bc}	21.33^{b}
HW40T	16.55^{abc}	16.35^{a}	14.61^{d}
HW50	16.30^{ac}	15.11^{bc}	19.01^{c}
HW50T	3.48^{e}	6.64^{e}	7.63^{e}
HW60	17.51^{ab}	12.52^{cd}	18.65^{c}
HW60T	12.14^{d}	12.38^{cd}	19.98^{bc}
T. ONLY	12.58^{d}	9.44d^{e}	12.29^{d}
CONTROL	19.58^{a}	19.19^{a}	31.44^{a}
S. CHECK	14.21^{bcd}	8.72^{e}	14.02^{d}

Note. Means on the same column followed by the same subscript are not significantly different (P< 0.05) based on Tukey's test.
LEGEND: 1. HW40. Hot water (40ºC) treatment for 5 min and priming in sterile water medium for 72 h.
2. HW50. Hot water (50°C) treatment 5 min and priming in sterile water medium for 72 h.
3. HW60. Hot water (60°C) treatment for 5 min and priming in sterile water medium for 72 h.
4. HW40T. Hot water (40°C) treatment for 5 min and priming in *T. viride* medium for 72 h.
5. HW50T. Hot water (50°C) treatment for 5 min and priming in *T. viride* medium for 72 h.
6. HW60T. Hot water (60°C) treatment for 5 min and priming in *T. viride* medium for 72 h.
7. T. ONLY. Water at ambient temperature for 5 min and priming in *T. viride* medium for 72 h.
8. CONTROL. Water at ambient temperature for 5 min and priming in sterile water medium for 72 h.
9. S. CHECK. Seed dressing with Mancozeb solution for 5 min priming in sterile water for 72 h.

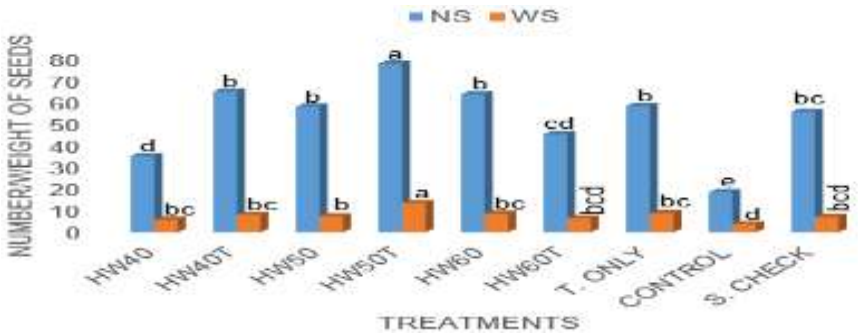

Figure 7. Effect of treatments on number and weight of cowpea seeds.
LEGENDS: NS: Number of seeds WS: Weight of seeds (g)
LEGEND: 1. HW40. Hot water (40°C) treatment for 5 min and priming in sterile water medium for 72 h.
2. HW50. Hot water (50°C) treatment 5 min and priming in sterile water medium for 72 h.
3. HW60. Hot water (60°C) treatment for 5 min and priming in sterile water medium for 72 h.
4. HW40T. Hot water (40°C) treatment for 5 min and priming in *T. viride* medium for 72 h.
5. HW50T. Hot water (50°C) treatment for 5 min and priming in *T. viride* medium for 72 h.
6. HW60T. Hot water (60°C) treatment for 5 min and priming in *T. viride* medium for 72 h.
7. T. ONLY. Water at ambient temperature for 5 min and priming in *T. viride* medium for 72 h.
8. CONTROL. Water at ambient temperature for 5 min and priming in sterile water medium for 72 h.
9. S. CHECK. Seed dressing with Mancozeb solution for 5 min priming in sterile water for 72 h.

significantly from the other treatments. CONTROL had the least number and weight of seeds. It produced 18.33 seeds that weighed 3.60 g. HW40T, HW50, HW60 and T. ONLY all produced seeds that were not significantly

Table 3. Effect of treatment on the severity of fusarium wilt on cowpea.

Treatments	Disease severity/ Weeks after transplanting		
	5	6	7
HW40	2.66^{bc}	2.88^{bc}	3.66^{a}
HW40T	3.33^{ab}	3.33^{ab}	2.16
HW50	2.83^{bc}	3.16^{ab}	3.33^{ab}
HW50T	1.33^{d}	1.66^{c}	1.33^{c}
HW60	2.83^{bc}	2.83	3.33^{ab}
HW60T	2.33^{bcd}	2.66^{bc}	3.33^{ab}
T. ONLY	1.83^{cd}	1.66^{c}	1.66^{c}
CONTROL	4.50^{a}	4.33^{a}	4.33^{a}
S. CHECK	2.33^{bcd}	1.66^{c}	2.33^{bc}

Note. Means on the same column followed by the same subscript are not significantly different (P < 0.05) based on Tukey's test.
LEGEND: 1. HW40. Hot water (40°C) treatment for 5 min and priming in sterile water medium for 72 h.
2. HW50. Hot water (50°C) treatment 5 min and priming in sterile water medium for 72 h.
3. HW60. Hot water (60°C) treatment for 5 min and priming in sterile water medium for 72 h.
4. HW40T. Hot water (40°C) treatment for 5 min and priming in *T. viride* medium for 72 h.
5. HW50T. Hot water (50°C) treatment for 5 min and priming in *T. viride* medium for 72 h.
6. HW60T. Hot water (60°C) treatment for 5 minu and priming in *T. viride* medium for 72 h.
7. T. ONLY. Water at ambient temperature for 5 min and priming in *T. viride* medium for 72 h.
8. CONTROL. Water at ambient temperature for 5 minu and priming in sterile water medium for 72 h.
9. S. CHECK. Seed dressing with Mancozeb solution for 5 min priming in sterile water for 72 h.

different in terms of their number. The number and weight of seeds from S. CHECK was poor, being the third lowest respectively (Figure 7).

DISCUSSION

Results from this study showed that cowpea seeds that were subjected to only hot water treatment had more numbers of germinated seeds compared to those that were bio-primed after hot water treatment. Hot water at 50°C (HW50) gave the best germination percentage. This finding is in agreement with reports from literature. Regulated heat has been reported to not only break dormancy and speed up germination rate, but also control certain seed-borne diseases of plants (Schmitt *et al.*, 2009; Tiryaki and Topu, 2014; Nandini and Kulkarni, 2015).

An interesting observation from the study was a reduction in the seedling length of seeds primed in *T. viride* medium after hot water treatment (HW40T, HW50T and HW60T) (Figure 3), compared to those in a sterile water medium. It is not very clear what may be responsible for this, because a similar trend was not observed in cowpea seeds primed in *T. viride* medium only (T. ONLY). Report from literature has however established the fact that *Trichoderma* spp. do colonize roots and inhabit intercellular spaces within them (Shoresh *et al*., 2010; Hermosa *et al*., 2012; Halifu *et al*., 2019). The colonization process may have interfered with root growth, especially after an initial stimulation by hot water treatment. Expectedly, all treatments that consisted of priming in *T. viride* medium after hot water treatment also had reduced seedling vigour. This was due to the reduced seedling length.

Results from the screen house study presented some interesting findings. In the first place, hot water treatment at 60°C (HW60) had poor seedling establishment. This trend was also observed in HW60T, which consisted of seeds that were primed in *T. viride* medium after hot water treatment at 60°C. The percentage of seedling establishment was even lower than HW60. Both treatments had poorer establishment percentage than CONTROL. Singh *et al*. (2019) had reported a similarly poor seedling growth of bell pepper whose seeds were subjected to hot water treatment at a temperature above 52°C. The exact impact of water at 60°C on cowpea seeds in this study cannot be stated categorically, since no test was designed to evaluate it. It is common knowledge, however, that germination, plant growth and

Table 4. Effect of treatments on cowpea plant height (cm).

Treatments	Plant height/ Weeks after transplanting		
	4	6	8
HW40	19.83^{de}	27.50^{bc}	36.00^{b}
HW40T	20.97^{bcd}	26.83^{c}	29.41^{e}
HW50	20.06^{cde}	25.70^{cd}	33.00^{d}
HW50T	23.61^{a}	30.20^{a}	38.41^{a}
HW60	21.55^{bc}	25.10^{cd}	33.83^{cd}
HW60T	18.58^{e}	21.75^{e}	34.41^{bcd}
T. ONLY	20.50^{bcd}	29.48^{ab}	33.85^{bcd}
CONTROL	19.43^{de}	23.26^{de}	24.80^{f}
S. CHECK	21.75^{b}	26.75^{c}	35.80^{bc}

Note. Means on the same column followed by the same subscript are not significantly different ($P < 0.05$) based on Tukey's test.
LEGEND: 1. HW40. Hot water (40°C) treatment for 5 min and priming in sterile water medium for 72 h.
2. HW50. Hot water (50°C) treatment 5 min and priming in sterile water medium for 72 h.
3. HW60. Hot water (60° C) treatment for 5 min and priming in sterile water medium for 72 h.
4. HW40T. Hot water (40°C) treatment for 5 min and priming in *T. viride* medium for 72 h.
5. HW50T. Hot water (50°C) treatment for 5 min and priming in *T. viride* medium for 72 h.
6. HW60T. Hot water (60°C) treatment for 5 min and priming in *T. viride* medium for 72 h.
7. T. ONLY. Water at ambient temperature for 5 min and priming in *T. viride* medium for 72 h.
8. CONTROL. Water at ambient temperature for 5 min and priming in sterile water medium for 72 h.
9. S. CHECK. Seed dressing with Mancozeb solution for 5 min priming in sterile water for 72 h.

Figure 8. (a). Leaf yellowing and premature defoliation in cowpea plant. (b). Wilted cowpea plant.

development are regulated by numerous enzymes that are extremely sensitive to temperature fluctuations. These enzymes are denatured at high temperature and become ineffective. It is very likely, therefore, that the enzymatic processes and their functions in cowpea seeds subjected to 60°C water treatment may have been disrupted. This view is supported by the findings of Black and Bewley (2000). They pointed out that high temperature has a damaging effect on enzymes and the DNA constituents of seeds. The impact of hot water on

Table 5. Effect of treatments on leaf production by cowpea plant.

Treatments	Number of leaves/ Weeks after transplanting		
	4	6	8
HW40	11.00^{bc}	22.20^{b}	16.50^{e}
HW40T	10.83^{bc}	22.50^{b}	19.83^{d}
HW50	12.50^{ab}	19.66^{c}	17.33^{e}
HW50T	13.60^{a}	24.66^{a}	27.45^{a}
HW60	10.83^{bc}	22.33^{b}	16.50^{e}
HW60T	8.50^{d}	19.33^{c}	22.33^{c}
T. ONLY	10.50^{c}	22.55^{b}	27.33^{a}
CONTROL	8.00^{d}	14.00^{d}	12.66^{f}
S. CHECK	11.00^{bc}	22.83^{b}	24.83^{b}

Note. Means on the same column followed by the same subscript are not significantly different (P< 0.05) based on Tukey's test.
LEGEND: 1. HW40. Hot water (40°C) treatment for 5 min and priming in sterile water medium for 72 h.
2. HW50. Hot water (50°C) treatment 5 min and priming in sterile water medium for 72 hours.
3. HW60. Hot water (60°C) treatment for 5 min and priming in sterile water medium for 72 h.
4. HW40T. Hot water (40°C) treatment for 5 min and priming in *T. viride* medium for 72 h.
5. HW50T. Hot water (50°C) treatment for 5 min and priming in *T. viride* medium for 72 h.
6. HW60T. Hot water (60°C) treatment for 5 min and priming in *T. viride* medium for 72 h.
7. T. ONLY. Water at ambient temperature for 5 min and priming in *T. viride* medium for 72 h.
8. CONTROL. Water at ambient temperature for 5 min and priming in sterile water medium for 72 h.
9. S. CHECK. Seed dressing with Mancozeb solution for 5 min priming in sterile water for 72 h.

the cowpea seeds was probably not strong enough to prevent germination altogether but became manifest as the seedlings of such seeds advanced in age and the need to deploy enzymes for growth and other activities became greater. This may have resulted in poor seedling establishment. The level of damage must have varied in the treated seeds though, as some were successfully established and grew to maturity. Another very important finding was that 50°C was the optimum temperature in terms of seed germination. Cowpea seeds primed in *T. viride* after hot water treatment at 50°C (HW50T) also gave the highest seedling establishment. Singh *et al*. (2019) reported a similar finding in bell pepper. None of the treatments exhibited complete resistance to fusarium wilt and disease incidence was recorded in all of them. HW50T was, however, the most tolerant as it consistently recorded the lowest incidence all through the data collection period. This observation may be due to two factors. Firstly, HW50 had the best seedling vigour. Seedlings with more vigour are better able to compete and are less susceptible compared to weak ones (Chadrashekhara *et al*., 2010; Nandini *et al*., 2016). They, therefore, stand better chances at preventing disease and overcoming infection. Report from literature suggests that such crops usually have better yields. Secondly, bio-priming of seeds has been shown to enhance disease resistance by activating the defense response in the seedlings and plants of such treated seeds (Nandini *et al*., 2016; Pathak *et al*., 2016), thereby bringing about reduced incidence and severity of targeted disease in such plants. This observation also explains the reason for the low disease severity in HW50T and the very high value of disease severity in CONTROL. A decline in the production of new leaves or outright reduction in the total number produced was recorded as from the 6th WAT. This was due to yellowing and wilting of leaves followed by premature defoliation (Figure 8a and b). All of which are symptoms of fusarium wilt disease of cowpea. This observation was most severe in CONTROL. The best yield parameters were obtained from HW50T. This is not unexpected, because the treatment had the tallest plant, produced the highest number of leaves with the least values for disease incidence and severity.

CONCLUSION AND RECOMMENDATION

HW50T recorded the lowest values for disease incidence and severity. It also produced most seeds with the highest weight. The treatment may be a promising option

in the management of fusarium wilt of cowpea, especially in an integrated approach. Further study, with particular attention on field trials, can be conducted for confirmation.

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