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EFFECT OF *Trichoderma harzianum* STRAINS IN ENHANCING DROUGHT TOLERANCE ON MAIZE UNDER WATER STRESS CONDITIONS IN SEMI-ARID REGIONS

H. S. Nzioki and D.L. Mutisya

Kenya Agricultural and Livestock Research Organization, KALRO-Katumani
P. O. Box 340. 90100. Machakos

ABSTRACT

Water stress is a major constraint to maize production in semi-arid Kenya. A field study was conducted at two sites in semi-arid Kenya to evaluate a new commercial product containing a mixture of *Trichoderma harzianum* Rifaistrains (TH-S) on enhancement of maize yield under drought conditions. Seed of an early maturing maize composite KDV1 was dressed with *T. harzianum* strains and planted under controlled irrigation conditions. A commercial strain of *T. harzianum* referred as EzySeed® was used for comparison as the industrial standard. The EzySeed® treatment had signficant higher numberof plants at harvest followed by the *T.harzianum* strains (TH-S) treatment. EzySeed out performed both TH-S and control on yield at the two test sites. The untreated control had the least number of plants. Maize treated with *T.harzianum* strains and EzySeed® had enhanced yield and root growth parameters in comparison to control indicating that an opportunity exists for further development of *T. harzianum* strains for management of drought stress on maize in semi-arid regions.

Keywords: *Trichoderma harzianum*, EzySeed®, Maize, Drought stress

INTRODUCTION

Trichoderama spp. are cosmopolitan soil fungi (Waksman, 1952) with a wide ecological adaptability (Hagn*et al.,* 2003; Roiger*et al.,* 1991).One hundred (100) *Trichoderma* species have been phylogenetically defined (Druzhinina*et al.,* 2006). In Kenya, 19 Trichoderma spp. have been isolated from several plant hosts and for land uses (Kung'u and Boa, 1997; Okoth*et al.,* 2007; 2009a;Maina *et al.,* 2015). Several rhizo sphere species of *Trichoderma*, commonly referred to as plant-symbiotic strains are used to enhance yield and to reduce plant diseases and

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abiotic stresses (Harman, 2011; Bae*et al.,* 2009; Mastouri *etal.,* 2009). The symbiotic strains colonize host roots and this results to enhanced tolerance to biotic and abiotic stresses by the host (Björkman *et al.,* 1998; Mastouri *et al.,* 2010). Plants with enhanced tolerance to biotic and abiotic stresses have enhanced root growth and nutritional status (Harman 2000, 2001; Harman *et. al.,*2004a;Yedidia *et al.,*2001), induced systemic resistance to diseases (Harman *et al.,* 2004b) and enhanced systems of reactive oxygen species (ROS) scavenging as well as for redox maintenance (Mastouri et al. 2010; 2012).

In Kenya, seven crop protection products containing *Trichoderma* spp. strains singly or in combination with other biological control micro organisms as active ingredients have been registered and are already in market (Woo *et al.,* 2014, *http://www.dudutech.com/product, realipm.com,* http://www.plant-health.co.za, http://www.sw-duenger.de). However, only two of these products, Eco-T® and Trithotech WP® are plant stimulators. Eco-T® contains *T. harzianum* Rifai *strain kd* and is registered for control of crop root diseases caused by Phytopthora, Pythium, Rhizoctonia, Sclerotiia and Verticilliumin fruit trees, vegetables, brinjals, cereals, pastures, brassicas, flowers, fibre crops, oil crops, trees, berries, tobacco and so lanaceous plants, and for enhancement and stimulation of plant growth under extreme conditions (http://www.plant-health.co.za). Trichotech WP®, contains *T. asperellum* as the active substance. It's registered for enhanced root development and management of botrytis blight, crown gall, Pythium, rhizoctonia, fusarium, sclerorinia and root and stem rots in cereals, vegetables, flowers and tea (http://www.dudutech.com/product). Presently, no data is available on evaluation of Eco-T® or Trichotech WP® on enhancement of drought tolerance in maize under semi-arid conditions in Kenya. Drought stress is a recurrent phenomenon in semi-arid regions of lower eastern and parts of upper eastern Kenya which receive annual rainfall of less than 850 mm. Rainfall patterns in these regions is bimodal, and its 60% reliable. The objective of this research was to evaluate *Trichoderma harzianum* strains, a commercial mixture of *T. harzianum* strains for enhancement of maize growth under water stress conditions in semi-arid Eastern Kenya.

MATERIALS AND METHODS

Seed pre-treatment- Seeds of open-pollinated maize variety commonly known as Kenya Dry land Variety 1 (KDV-1) was used. It was sourced from KALRO Seed Unit (KSU) at Katumani Research Centre. *T. harzianum* strains (TH-S) and green dye (TH-A), all in liquid formulation were supplied by Kenya Biologics Ltd. EzySeed® was used as the industrial standard. It was supplied by Kenya Biologics Ltd in powder formulation. Coating maize seed with *T. harzianum* strains (TH-S) and EzySeed® was conducted at the time of planting. The seeds were sown immediately after coating. The contents of both tubes of TH-A (30 ml solution of green dye) and

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TH-B (30 ml solution of *T. harzianum* strains) were shaken and then poured into a 500 ml plastic measuring cylinder. Sterile distilled water was added to make total volume to 200 ml. Two kilogram of maize seed to be coated with *T. harzianum* strains was put in 8 litre surface-sterilized clear plastic container. The 200 ml mixture of TH-A and TH-B was slowly sprinkled on the 2 kg maize seed. Little sterile distilled water was sprinkled on the seed to make it wet. The lid of the 8 litre clear plastic container was replaced and the contents were slowly shaken by hand until there was an even coating of the product on all seeds as was indicated by appearance of equal green colouration on all seeds. Similarly two kilogram of maize seed was placed in similar 8 litre surface sterilized clear plastic container for the EzySeed® product (of black colouration) same procedure of mixing followed. For the control two kilogram of maize seed was placed in similar 8 litre surface sterilized clear plastic container. Enough sterile distilled water was sprayed on the seed to wet it. The lid of the container was replaced and the contents shaken until all the seeds were wet.

Plot establishment- Trials were planted in two sites in KALRO Kiboko sub-station (02° 7' 51" S, 037° 25 57" E, 934 m asl) in Makueni County and in a farmer's field at Kabaa irrigation scheme (01° 15' 0" S, 037° 25' 60" E, 1248m asl) in Machakos County. The Kiboko site was planted on 12th August 2015 while the Kabaa site was planted on 17th July 2015. Maize was planted at the recommended spacing of 30 cm within rows and 90 cm between rows. Each plot/treatment consisted of eleven 10 m long maize rows in Kiboko, and ten 10 m long rows in Kabaa. The treatments were arranged in a completely randomized block design, each replicated six times. The soil type in Kabaa was sandy clay loam, while in Kiboko it was sandy clay. The plots were treated in the same manner as commercially grown maize. Fertilizer application of DAP at rate of 20kg/haat planting was carried out, later CAN top dressing was done at rate 20 kg/ ha. Field plot weeding was three times during crop development. Sprinkler irrigation was used at Kiboko. The plots were irrigated immediately after planting and subsequently three times per week until germination and subsequently once per week for 5 weeks. Irrigation was stopped when 100 % of the plants had flowered and at least 50 % were at silking stage. Furrow irrigation was applied at Kabaa. After ploughing and harrowing, the land was first irrigated for four consecutive days (6 hrs per day) to wet the soil. Planting was then conducted two days later after wetting the soil. Water was then re-applied 3 days after planting and subsequently once per week until the maize flowered. Irrigation was stopped at 50 % flowering (42 days after planting) and less than50 % silking.

Data collection and analysis- The following data was collected: germination %, stand count after thinning, pest incidence at vegetative, flowering and physiological maturity stages. During harvesting, the following data was recorded from the harvest area: plant stand at harvest, number

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of ears at harvest, and field weight (weight of maize cob with grains). The harvest area consisted of five middle maize rows of each plot. Two plants from the edges of each row in each plot were not harvested (due to border effect). The grain weight from each plot was measured after shelling and drying the grain to a moisture content of 12-13%. To compare root development, five root samples were picked at random from a batch of 25 plants from the harvested area in each plot. The total number of secondary roots and root length (cm) from the five secondary roots samples (from five plants per plot) were recorded. Data was analysed by statistical analysis systems (SAS, PROC GLM) for significant difference by Student Neumann Keuls Test (SNK). However, first position ranking (%) of maize yield and root growth parameters in the various treatments in both sites was calculated by a simple formula: Number of times parameter in rank no.1 x 100
Total number of parameters

RESULTS

Number of plants and ears at harvest- The EzySeed® treatment had signficant ($p< 0.05$)higher numberof plants at harvest followed by the *T.harzianum*strains (TH-S) treatment (Table 1).The untreated control had the least number of plants. The number of ears at harvest were more in the *T. harzianum* strains treatment than in EzySeed® and untreated control treatments.Overall maize plants at Kiboko plot performed significantly ($p< 0.05$) better in terms of plant establishment and ears setting than Kabaa.

Table 1: Number of plants and ears at harvest at two different sites of eastern Kenya in 2015

	No. plants				No. plant ears			
Site	EzySeed ®	TH-S	Control	*P*	EzySeed ®	TH-S	Control	*P*
Kiboko	119a^{A}	118ab^{A}	117b^{A}	0.0331	93a^{A}	92a^{A}	87b^{A}	0.0004
Kabaa	99a^{B}	76c^{B}	88b^{B}	< 0.0001	50a^{B}	29c^{B}	39b^{B}	< 0.0001
SE	1.1	2.1	1.4		2.0	0.4	2.5	
P	0.0019	0.0017	0.0016		0.0008	< 0.0001	0.0050	

Different letters within rows indicate significant ($P< 0.05$) difference of fungus effect on plant establishment and number of ears at each site (Student Neumann Keuls Test) at 5% level (df = 2, 17). Likewise different superscript uppercase letters indicate significant ($P< 0.05$) difference of fungus effect to plant parameters between sites (df = 1, 17).

Field weight and grain weight- *Trichoderma harzianum* strains (TH-S) treatment had insignificant ($p> 0.05$)ear biomass relative to EzySeed® and control treatments (Table 2). Grain yield wa not significant among treatments at Kiboko while EzySeed® treatment had relatively higher tonnage compared to the other treatments. Between the two site plots yield was significantly higher at Kiboko at 1.2 t ha^{-1} while at Kabaa it was twice lower. The control

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treatment ranked last in ear biomass and grain yield respectively. Likewise, plot location demonstrated insignificant ear biomass at the two sites. A significant ($p < 0.05$) difference on ear biomass and grain weight wa noted between the two site plots on *T. harzianum* (TH-S) and control treatments.

Table 2: Plant ear biomass and yield (t ha^{-1}) at two different sites eastern Kenya in 2015

	Ear biomass (kg)				Grain yield t ha^{-1}			
Site	EzySeed ®	TH-S	Control	*P*	EzySeed ®	TH-S	Control	*P*
Kiboko	8.8a^{A}	8.7a^{A}	8.5a^{A}	0.9335	1.2a^{A}	1.2a^{A}	1.1a^{A}	0.5776
Kabaa	6.0a^{A}	4.0b^{B}	3.7b^{B}	0.0272	0.6a^{B}	0.5b^{B}	0.5b^{B}	0.0123
SE	1.7	0.5	0.2		0.4	0.2	0.3	
P	0.2731	0.0014	0.0006		0.0145	0.0035	0.0039	

Different letters within rows indicate significant ($P< 0.05$) difference of fungus effect on plant ear biomass and grain weight at each site (SNK Test) at 5% level (df = 2, 17). Likewise different superscript uppercase letters indicate significant ($P< 0.05$) difference of fungus effect to plant parameters between sites (df = 1, 17).

Root length and number of roots-

On root length, no significant ($p > 0.05$) difference was noted at Kiboko while *T. harzianum* (TH-S) had significantly ($p< 0.05$) higher length at 12.9cm in comparison to 5.3cm for both control and EzySeed® at Kabaa. At Kiboko TH-S led with highest number (17.3) of maize secondary roots while control and EzySeed® were at 11.0 and 11.1 respectively. EzySeed® had 14.8 roots per plant in comparison to 12.1 for TH-S and control respectively at Kabaa.

Table 3: Root length (cm) and numbers in two different sites in eastern Kenya in 2015

	Root length (cm)				No. plant roots			
Site	EzySeed ®	TH-S	Control	*P*	EzySeed ®	TH-S	Control	*P*
Kiboko	4.9a^{B}	7.4a^{B}	4.3a^{B}	0.1361	11.1b	17.3a^{A}	11.0b^{A}	0.0153
Kabaa	5.3b^{A}	12.9a^{A}	5.3b^{A}	0.0019	14.8a	12.1b^{B}	12.1b^{A}	0.0356
SE	0.1	1.4	0.1		2.1	2.4	0.1	
P	0.0263	0.0123	0.0865		0.0903	0.0459	0.2314	

Different letters within rows indicate significant ($P< 0.05$) difference on plant root length and grain weight at each site (SNK Test PROC GLM) at 5% level (df = 2, 17). Likewise different superscript uppercase letters indicate significant ($P< 0.05$) difference of fungus effect to plant parameters between sites (df = 1, 17).

Ranking of maize yield and root growth parameters- When the maize yield and root growth parameters were ranked, TH-S and EzySeed® performed equally better than the control in Kiboko site (Table 4). In Kabaa site, EzySeed® ranked ahead of TH-S and control, and TH-S

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ranked ahead of control. None of the maize yield and root growth parameter(s) were ranked No. 1 in the control treatment compared to TH-S and EzySeed® treatments.

Table 4: Ranking of maize growth parameters at Kiboko and Kabaa in eastern Kenya in 2015

	Kiboko			Kabaa		
Parameter	TH-S	EzySeed®	Control	TH-S	EzySeed®	Control
NP	2	1	3	3	1	2
NE	1	3	2	3	1	2
EB	1	3	2	3	1	2
GW	1	3	2	2	1	2
% in rank 1	50	50	0	33.3	66.7	0

NP = number of plants, NE = number of ears, EB = ear biomass, GW = grain weight

DISCUSSION

In the present study *T. harzianum* strains (TH-S) and EzySeed® positively influenced growth and development of maize under water stress conditions compared to control treatment. The study provides indicative evidence that in most of the cases, the EzySeed® and TH-S seed treatments performed relatively better than the control in terms of increasing maize yield and root growth parameters in Kiboko and Kabaa sites respectively. Soil temperature, soil moisture, soil pH, soil organic matter and type of host plant (Carreiro and Koska, 1992;Okoth*et al.,* 2009b), and carbon and nitrogen sources determine the ability of *Trichoderma* spp. to colonize host plants. *Trichoderma* conidial concentrations and organic and inorganic amendments also determine optimal performance of the fungus (Harman *et al.,* 1981; Ali *et al.,* 2012).

Trichoderma spp. can grow in a wide temperature range, as low as 0 °C for *T. polysporum* and as high as 40 °C for *T. koingii* (Tronsmo and Dennis, 1978). *T. hamatum* prevented diseases of pea caused by *R. solani* and *Pythium spp*. respectively at soil temperatures of between 17 and 34 °C. The maximum growth and sporulation of *T. viride* occurred at a pH of 4.5 to 5.5, and temperatures of 20-37 °C (Jayaswal*et al.,* 2003).Although measure of the soil temperature at the two sites was not taken during planting, mean maximum and mean minimum air temperature in the two sites are between 20-25° Cday and 10 -15 °C night, respectively. Acidic soils favour growth of *Trichoderma* strains relative to alkaline soils (Benitez*et al.,* 2004, Raut*et al.,* 2013, Rousky*et al.,* 2009; Schubert*et al.,* 2009).Populations are more abundant in moist soil especially in humid litter (Danielson and Davey, 1973a). Kredic (2003) found a linear correlation between water potential and colony growth rate at both 25 and 10 °C with growth rates at higher temperature and water potential. The effect of carbon and nitrogen source on growth and sporulation of some *Trichoderma* spp. was studied by Jayaswal*et al.* (2003); Ali *et al.* (2012) and Monga *et al.* (2001) among others. Growth and sporulation of *T. viride* was best when carbon sources were sucrose, peptone and trehalose, and nitrogen sources were ammonium forms (Jayaswal*et al.,* 2003). Maximum sporulation of *T. harzianum* occurred when sucrose and glucose were used as carbon sources and glycine was used as the nitrogen source (Monga, 2001). *T. hamatum* worked best on control of diseases of pea and radish caused by *Pythium spp*.or *Rhizoctoniasolani* respectively when soil temperatures were 17-34 °C and seeds were inoculated with a conidial suspension with a concentration equal to or greater than 10^6/ml (Harman *et al.,* 1981). At a pH of 5.5 and under laboratory conditions, *T. harzianum* produced the best dry weight and the best antagonistic potential (Ali *et al.,* 2012). Inoculating *T. harzianum* into the soil as mycelial fragments grown on rice husks and adding chitin and sodium nitrate as carbon and nitrogen sources resulted in better survival and proliferation, and induced growth and best antagonistic potential (Ali *et al.,* 2012). The requirements of different bio agents used against many soil borne plant pathogens have been worked out (Jackson *et al.,* 1991a; Aube and Gagman, 1969; Danielson and Davey, 1973b, 1973c).The response of *T.harzianum* strains to maize performance depends on the maize genetic make-up (Harman *et al.,* 2004a). Some maize inbred lines respond negatively to some strains (Harman, 2006). *T. harzianum* Strain T22 reduced maize yields with the hybrid Sgi x Sgi 861 but produced strong positive growth response in maize inbred line Mo17.

CONCLUSION

The present results show that opportunities exist for application of *T. harzianum* strains to promote growth and enhance yield of maize under water stress conditions in semi-arid Kenya. Future studies should focus on (i) screening commercial maize varieties for their responsiveness to *T. harzianum* strains, (ii) studies on effect of organic and inorganic amendments on *T. harzianum* to promote maize yield under drought conditions, (iii) studies on effect of soil amendments responsiveness of *T. harzianum* to maize yield, and (iv) studies on effect of *T. harzianum* strains to maize yields when applied across different soil amendment regimes under water deficit conditions. Future studies should incorporate equipment for measuring when water deficit conditions occur in maize, when to apply water and when to stop applying water. Stoppage of water application to maize at the right maize stage may create a conducive environment for *T. harzianum* strains in the maize rhizosphere to induce drought tolerance in maize.

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