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EFFECT OF *TRICHODERMA HARZINUM* FERMENTED BROTH ON SEED GERMINATION AND SEEDLING GROWTH OF MAIZE (*ZEA MAYS*)

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ABSTRACT
Trichoderma harzianum is well known for its biocontrol activities, but resent studies also shows that it promotes plant growth, so investigation was carried out to study effect of *Trichoderma harzianum fermented* broth on seed germination, seedling growth and biomass production of maize (*Zea mays*) . *Trichoderma spp.* were isolated from the soil and fermentation is carried out on modified glucose media .the fermented broth was filtered and filtrate was used as fermentation product. For analysis purpose 0%, 50%,100% fermented broth used. The fermented broth show significant different in all studied parameters in maize. It indicates that Trichoderma produces extracellular plant growth regulators and release them into fermented broth which promote seed germination and seedling growth in maize.

KEYWORDS: Fermentation, Plant growth regulators, *Trichoderma, Zea mays.*

INTRODUCTION

Maize, wheat and rice make up to 85% of global cereal production. They are often the only source of nutrition in developing and underdeveloped countries. In many parts of Latin America, Africa and Asia, maize is the major staple food and often the only source of protein. Additionally, 78% of total maize production goes into livestock feed in developed countries.

Trichoderma spp. is free-living fungi that are common in soil and root ecosystems. It has gained immense importance since last few decades due to its biological control ability against several plant pathogens. *Trichoderma spp.* are being employed widely in plant agriculture, both for disease control and yield increases (Harman *et al.*,2006: Chang *et al.*,1986). In addition to the ability of *Trichoderma spp*. to attack or inhibit the growth of plant pathogens directly, recent discoveries indicate that *Trichoderma spp.* have evolved multiple mechanisms that result in improvements in plant resistance to disease and plant growth and productivity (Harman *et al*., 2004; Vinale *et al.*, 2008).These new findings are dramatically changing our knowledge of the mechanisms of action and uses of these fungi.

More recently, many workers observed that *Trichoderma* have diverse antifungal mechanisms and ability to promote plant growth, It stimulate plant growth in cucumber, cabbage, lettuce, potato, tomato, carrot, beans and peas (Ousley *et al.*, 1994 ; Khan *et al.*, 2004; Rabeendran, 2000; Yossen *et al.*, 2003). Presumed mechanisms involved in the stimulation of plant growth by Trichoderma include interactions with plant roots similar to Mycorrhizae, in which *Trichoderma* penetrates and colonizes root tissues without eliciting specific defense responses against the colonizing strain (Yedidia *et al.*, 1999).Other possible explanations of this phenomenon include: control of minor pathogens leading to stronger root growth and nutrient uptake (*Ousley et al.*, 1993), secretion of plant growth regulatory factors such as phytohormones (Chang *et al.*, 1986) and release of soil nutrients and minerals by increased saprophytic activity of *Trichoderma* in the soil (Ousley *et al.*, 1993). This study was carried out with the objective to evaluate the effect of *Trichoderma* fermented broth on seed treatments on the germination and seedling quality of maize.

MATERIALS AND METHODS

Isolation and identification of strains of *Trichoderma.*

The strains of *Trichoderma spp* .was isolated from soil samples of Sangamner area. .
For isolation of *Trichoderma* strains, a serial dilution technique was followed.. one milliliter of each solution was pipetted onto a Rose Bengal Agar (RBA) (J C Ottow and H Glathe 1968) plate and incubated at 28 °C for 1 week. The culture plates were examined daily and each colony that appeared was considered to be one colony forming unit (cfu). After enumeration of cfu, individual colonies were isolated from the same plates and each uncommon colony was re-isolated onto a fresh potato dextrose agar (PDA) plate. Distinct morphological characteristics were observed for

identification, and the plates were stored at 4 °C. Two techniques, visual observation on Petri dishes and micro-morphological studies in slide culture, were adopted for identification of *Trichoderma harzianum* species. For visual observation, the isolates were grown on PDA agar for 3-5 days. For micro - morphological studies, a slide culture technique was used. Examination of the shape, size, arrangement and development of conidiophores or phialides provided a tentative identification of *Trichoderma harzianum.*

Maintenance of pure culture of isolated organism:
Colony was purified by transfer and retransfer on fresh PDA plates, pure culture will be maintained on PDA slants and slants was maintained in incubator at optimum temperature of 28^0C.

Fermentation of *Trichoderma harzianum* -
The subculture of the isolated strain of *Trichoderma harzianum* was done at 28°C on potato-dextrose agar slants and incubated for six days. Inoculums was prepared from six days old slats and directly inoculated in to flasks. The inoculum flasks contained 100 ml sterile medium (Ammonium chloride - 3.5 g per Lit. Sucrose - 30 g per Lit n Ammonium sulphate - 2 g per Lit. Calcium carbonate - 7 g per Lit).The inoculated flasks with 50 ml of medium, were incubated at 28°C for 24 to 48 hrs. in a mechanical agitator. This inoculums was used at a concentration of 1.5% for flasks fermentations. The flask fermentations were carried out in three different 500 ml conical flasks containing 200 ml. of Glucose Media : Glucose - 40 g/Lit ; Ammonium tartarate – 9.5 g/Lit ; Monopotassium phosphate - 2.0 g/Lit ; Potassium sulphate – 0.6 g/Lit ; Magnesium sulphate - 0.2 g/Lit ; Ammonium chloride – 3 g/Lit. and incubated at 28°C on a rotary shaker at 200 rpm. The fermentations, in general, were allowed to proceed for 10 days. The temperature was maintained at 28°C during the entire fermentation period while the initial pH was 5.0 to 5.8. After 10 days the broth is filtered with filter paper of known quality and the filtrate used as fermented broth.

Seed germination and seedling growth-
Maize seeds were surface sterilized with 0.1% mercuric chloride $(HgCl)_2$
and washed with distilled water, then seeds are socked in 0%,50% and 100% concentrated fermented broth. Presoaked seeds was placed equispacially in sterilized petriplates, lined with filter paper soaked with different concentrations of fermented broth and distilled water. These petriplates were irrigated with different concentration of effluent uniformly. Number of seed germination was counted on 4th day and total germination percentage was calculated. Seedlings growth was analyzed on 5 day in terms of shoot and root length and fresh weight and dry weight measured on 15 th day of sowing. The data were put to statistical analysis. The SD values are given in Table. Significance of treatment effects was tested by standard deviation.

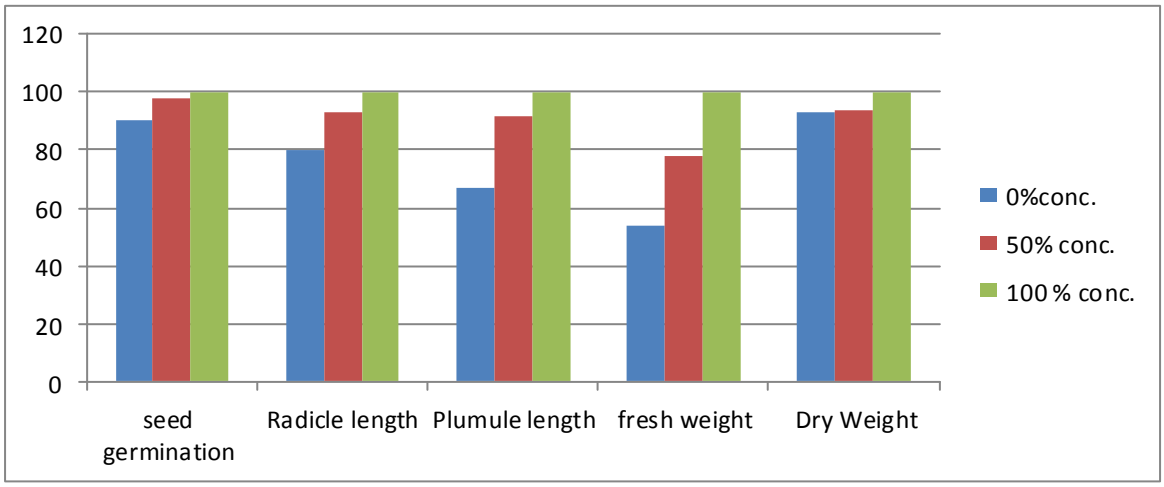
RESULTS AND DISCUSSION
Trichoderma harzianum fermented broth was analyzed for its properties which show change in some properties which includes color is changed from pale yellow to golden yellow and odor became fishy .

Table : Effect of *Trichoderma harzianum* fermented broth on seed germination and seedling growth of maize.

	DAS*	Effluent concentration (%)		
		00	50	100
Seed germination %	4	74 ± 1.33	80±1.83	82±4.07
Length of radical (cm)	5	6.25±0.20	7.24±0.63	7.70±0.57
Length of plumule (cm)	5	5.50±0.32	7.68±0.53	8.28±0.65
Seedling biomass				
Fresh weight (g/seedling)	15	1.08±0.30	1.58±0.35	1.94±0.38
Dry weight (g/seedling)	15	0.110±0.010	0.112±0.013	0.118±0.012

*DAS-Days after sowing. ± Standard deviation

The pH of broth change from 7 to 5.5. Which indicates that *Trichoderma harzianum* produce some extracellular chemicals which results in change in the pH of broth. The effect of different concentration of *Trichoderma harzianum* fermented broth was analyzed on seed germination and seedling growth and biomass production. for the purpose of analysis 0%,50% and 100% conc. fermented broth was used .as the concentration of fermented broth increases it show positive effect on all studied growth parameters (table). Maximum promoting effect was recorded at 100% concentration.



Graph 1. Relative effect of *Trichoderma harzianum* fermented broth on seed germination, early growth and biomass yield of Maize seedlings.

DISCUSSION

Trichoderma harzianum fermented broth shows significant increase in all studied parameters in maize .Possible conclusion for increase in seed germination and seedling growth is *Trichoderma harzianum* produces some growth regulators and releases them into external medium which shows significant increase in studied parameters.

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