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Effect of Micro Algae on *Rhizoctonia solani* and Botanicals on *Curvularia* spp. of Finger Millet (*Eleusine coracana* L.)

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A B S T R A C T

Finger millet (*Eleusine coracana* L.) is a member of eight minor millets group, constitutes about 81% of minor millets produced in India. The crop is affected by sheath blight caused by *Rhizoctonia solani* and leaf spot caused by *Curvularia* spp. Different doses of microalgae were applied @ (1.5, 2.5, 3.5, 4.5 and 5.5kg/acre) with irrigation at critical growth stages of crop (Vegetative phase, Flowering phase and Maturity phase) and observations were recorded on plant growth parameters and per cent disease incidence of *R. solani*in leaf sheath. *In-vitro* effect of different botanicals (Trumphet leaf extract, Trumphet flower extract, Bryophyllum leaf extract, Ashok leaf extract and Moringa leaf extract) at different concentrations (5 and 10%) were screened on radial growth of *Curvularia* spp. which was isolated from the leaf of Finger millet. Results shows that, the application of microalgae of all the doses significantly reduced the per cent disease incidence of *R. solani* as compared with control at 60 and 90 DAT of microalgae. *In-vitro* effect of the botanicals on radial growth of *Curvularia* spp. was recorded after 96 hrs of incubation and the result showed that, among the treatments, Ashoka leaf extract at 5% and 10% significantly inhibited the percentage mycelial growth with 60.60% and 81.96% which was significant over other botanicals. All the botanical extracts significantly reduced the radial growth of *Curvularia* spp. from control.

Introduction

In India, finger millet is cultivated over an area of 1.19 million hectares with a production of 1.98 million ton giving an average productivity of 16.61 q/ha. Karnataka accounts for 56.21and 59.52% of area and production of finger millet followed by Tamil Nadu (9.94% and 18.27%), Uttarakhand (9.40% and 7.76%) and Maharashtra (10.56% and 7.16%), respectively (Sakamma *et al*., 2018).

Finger millet crop is affected by several biotic stresses of which diseases like Finger millet blast, Sheath blight (*Rhizoctonia solani*) and *Curvularia* leaf spot cause qualitative and quantitative reduction in yield. Sheath blight of little millet incited by *Rhizoctonia solani* (Kuhn.) is one of the emerging malady in

successful cultivation of little millet Akhtar *et al.* (2009) reported wide spread occurrence of sheath blight of maize caused by *Rhizoctonia solani* in Jharkhand with disease severity ranging from 30.30 to 80.46%. Anonymous (2013) reported sheath blight incidence in little millet entries at Rewa (2.3 to 40.4%), Ranchi (0.0 to 34.5%) and Vizianagaram (0.0 to 60.0%).

The microalgae filtrate exhibited an inhibitory activity against the fungal tested organisms. Significant stimulating effects were produced on the tomato seeds which previously soaked in *C. minutus* culture filtrate, in retarding the *Phythium spp*. symptoms (Ibrahim *et al.*, 2008). Extracts from sea weeds (microalgae) sprayed on plants have been reported to reduce the incidence of *Botrytis cinera* (gray mold) on strawberries, *Erysiphe polygoni* (powdery mildew) on turnips, and damping-off of tomato seedlings (Kulik, 1995). The Cyanobacterial and algal extracts, the algal extracts used in the study showed higher efficiency towards antimicrobial activity. Microalgae are able to produce biomass that might be used in different sectors such as: Fuel, food, animal feed, pharmaceutical and crop productions. Regarding crop productions, microalgae contain high levels of macronutrients and micronutrients essential for an optimal crop growth and development.

Plants produce numerous secondary plant metabolites that are insignificant for growth and developmental processes (Rosenthal *et al.*, 1991) act against microbial pathogens on the basis of their toxic nature (Schafer *et al.*, 2009). Exploration of plant-based pesticides to control post-harvest losses is one of the feasible methods, lead to ecofriendly use of natural products, act as rich source of natural compounds exhibiting many fungicidal and other properties with least side effects. Antifungal activity of plant extracts may be more effective than some commercial synthetic fungicides natural occurring substances in plants with anti-microbic properties (Tamuli, 2014). Therefore, it has become necessary to adopt ecofriendly management practices for plant health management and better yield. In the present review different *in-vitro* studies are discussed to control plant pathogens.

Considering the above mentioned facts, a study entitled "Effect of microalgae on *Rhizoctonia solani* and botanicals on *Curvularia* spp. of Finger millet (*Eleusine coracana* L.)

Materials and Methods

Isolation and Identification of *Rhizactonia solani* from leaf sheath of finger millet

Isolation of *R. solani*

Infected leaves and sheath of Finger millet were washed first in water and thenin mercuric chloride ($HgCl_2$) to disinfect the surface of leaves. After that, leaves were cut into small pieces and transferred to the Petri plates containing PDA (four pieces per plate) and plates were incubated at 25 ± 2^0C for 3-4 days in inverted position. After 3-4 days, colonies of the fungus appeared and slides were prepared (Tuite, 1969). The fungus was purified through hyphal tip/single sclerotial method (Rangaswami and Mahadevan, 2004).

Identification of *R. solani*

The pathogen (*R. solani*) isolated in PDA was produced white mycelial growth at first but later on it turned into light cream color to yellowish cream color. The mycelium was septate and hyaline. Further the mycelium aggregated and formed the sclerotia with respected color and pattern of distribution (Butler, 1957).

Isolation and identification of *Curvularia* spp. from leaf spot of finger millet

Isolation of *Curvularia* spp

The infected leaves was brought and cut into small pieces, surface sterilized with mercuric chloride for 15-30 seconds, rinsed with three changes of sterile distilled water to remove disinfectant and bottled dry. The sterilized pieces were plated on Potato Dextrose Agar (PDA) medium in petri plates under aseptic conditions and incubated at 25^{o}C for 2 weeks. For obtaining sufficient quantity of inoculums, pure cultures was obtained by sub culturing.

Identification of *Curvularia* spp

The pathogen (*Curvularia* spp.) isolated in PDA was produced black color mycelial growth. The microscopic structure of conidia observed was dark/pale brown conidia with 3/4 septa and the conidia was curved and prominence of geniculate growth pattern was observed. The central cell is typically darker and enlarged compared to the end cells in the conidium. The swelling of the central cell usually gives the conidium a curved appearance (Keys identified based on literature of (Benoit and Mathur, 1970).

Preparation of plant extracts

The freshly selected Trumpet leaves, Trumpet flowers, Bryophyllum leaves, Ashoka leaves and Moringa leaves were collected and washed thoroughly with clean water and dried. Plant leaves were grounded in a pestle and mortar by adding same proportion of sterilized distilled water in weight by volume method (1:1 w/v). The extract was filtered through double layered muslin cloth and the filtrate were added to PDA media according to the required concentrations for poison food technique (Gurjar *et al*., 2012).

Poison food technique

Five-millimeter diameter disc of *Curvularia* spp. was kept at the centre of each Petri plate containing botanicals of required concentration dissolved in PDA. Three replications were maintained. The plates were incubated at 27±1^{o} C for ten days and colony diameter was recorded. Per cent inhibition of mycelial growth was calculated by using the formula given by Vincent (1947):

$$I = \frac{(C - T)}{C} \times 100$$

Where
C = Mycelium weight in control.
T= Mycelium weight in treatment.

Results and Discussion

In vitro evaluation ofbotanicals against *Curvularia* spp

The results presented in table 1 and depicted in plate no (1) and figure (1) revealed that all the botanicals tested were significantly effective in inhibiting the growth of pathogen over control. The results indicated that increase in percent inhibition was variably in proportion to increase in the concentration (5 and 10%) of the plant extracts. Among different plant extracts tested Ashoka leaves extract at 5 and 10% showed maximum inhibition followed by Bryophyllum leaf extract, Trumpet flower extract, Moringa leaf extract and minimum inhibition was showed inTrumpet leaf extract.

However, at 5% concentration, all the treatments were significant over control. Among the treatments (T_4 and T_2), (T_2 and T_3) and (T_3 and T_5) were found non-significant to each other.

At 10% concentration, all the treatments were significant over control. Among the

treatments, (T_3 and T_2), (T_2 and T_5) and (T_5 and T_1) were non significant and statistically at par with each other.

Effect of Micro alage at different doses on *R. solani* disease incidence of Finger millet at 60 and 90 DAT

Perusal of data in table 2 (Fig 2) reveals that Microalgae 5.5kg/acre at 60 and 90 DAT has significantly reduced the per cent disease incidence (20.11% and 26.13%) as compared with Microalgae 4.5kg/acre (22.65% and 29.50% respectively), Microalgae 3.5kg/acre (24.41% and 34.71% respectively), Microalgae 2.5kg/acre (26.10% and 39.07% respectively), Microalgae 1.5kg/acre (27.40% and 41.60% respectively), and control (28.70% and 47.72% respectively).

However, at 60 DAT the treatments (T_2 and T_1) were found non-significant and statistically at par with each other, at 90 DAT all the treatments were found significant over control.

Plant height (cm)

Perusal of data in table 3 (Fig 3) reveals that Microalgae 5.5kg/acre at 30, 60 and 90 DAT has significantly increased the plant height (25.90,77.71 and 112.80), as compared with other treatments i.e., Microalgae 4.5kg/acre (23.89,75.33 and 109.66), Microalgae 3.5kg/acre (23.11,72.67 and 103.16), Microalgae 2.5kg/acre (21.34,70.51 and 101.33), Microalgae 1.5kg/acre (19.67, 68.91 and 99.10), and untreated check (17.30, 66.57 and 91.54).

However, at 30 DAT the treatments (T_3 and T_4) were found non-significant and statistically at par with each other, At 60 and 90 DAT all the treatments were found significant over control.

Yield components of finger millet

Number of Spikelets

Data recorded in Table 4 showed that, among all the treatments, Number of spikelets were significantly increased in Microalgae @5.5kg/acre (6.67) followed by Microalgae @ 4.5kg/acre (6.33), Microalgae @3.5kg/acre (6.0), Microalgae @2.5kg/acre (5.67) and Microalgae @1.5kg/acre (5.33) in comparison of control (4.67). However, all the treatments were significant over control. Among the treatments (T_0, T_1, T_2 ,T_3 and T_4), (T_1, T_2, T_3, and T_5), (T_2, T_3,and T_4), (T_3, T_4 and T_5) and (T_4 and T_5) were found non-significant to each other.

Length of spikelets

Data recorded in Table 4 showed that, among all the treatments, Length of Spikelets (cm) were significantly increased in Microalgae @5.5kg/acre (11.57) followed by Microalgae @4.5kg/acre (9.93), Microalgae @3.5kg/acre (9.47), Microalgae @2.5kg/acre (8.40) and Microalgae @1.5kg/acre (8.00) in comparison of control (7.30). However, all the treatments were significant over control. Among the treatment (T_0 and T_1), (T_1 and T_2) and (T_3 and T_4) were found non-significant to each other.

1000 grain weight

Data recorded in Table 4 showed that, among all the treatments, 1000 grain weight (gm) were significantly increased in @ Microalgae @5.5kg/acre (3.65) followed by Microalgae @4.5kg/acre (3.41), Microalgae @3.5kg/acre (3.23), Microalgae @2.5kg/acre (3.02) and Microalgae @1.5kg/acre (2.82) in comparison of control (2.60). However, all the treatments were significant over control.

Table.1 *In vitro* effect of botanicals on radial growth (mm) of *Curvularia* spp. at different concentrations

Treatment no.	Treatment name	Radial growth (mm) of the three replicates mean after 96hours			
		5% conc.	Inhibition %	10% conc.	Inhibition %
T_0	Control	85.00	0	85.00	0
T_1	Trumpet Leaf extract	50.66	23.24	32.66[c]	61.57
T_2	Trumpet flower extract	30.50[ab]	53.78	26.66[ab]	68.63
T_3	Bryophyllum leaf extract	32.66[bc]	50.51	25.00[a]	70.58
T_4	Ashoka leaf extract	26.00[a]	60.60	15.33	81.96
T_5	Moringa leaf extract	36.66[c]	44.45	31.00[bc]	63.52
SEd(±)		2.45	-	2.30	-
CD (at 5%)		5.35	-	5.03	-

Means with similar letters represents non-significant to each other at 5% level.

Table.2 Effect of Microalgae on Disease incidence (%) at different DAI Sheath blight in Fingermillet

Treatment details	Disease incidence(%)	
	60DAT	60DAT
T_0 Control	**28.70**	**47.72**
T_1 (Microalgae 1.5kg/acre)	**27.40[a]**	**41.60**
T_2 (Microalgae 2.5kg/acre)	**26.10[a]**	**39.07**
T_3 (Microalgae 3.5kg/acre)	**24.41**	**34.71**
T_4 (Microalgae 4.5kg/acre)	**22.65**	**29.50**
T_5 (Microalgae 5.5kg/acre)	**20.11**	**26.13**
SEd(±)	**0.59**	**0.67**
CD (5%)	**1.32**	**1.50**

Means with similar letters represents non-significant to each other at 5% level
*Mean of Three Replications.

Table.3 Effect of Microalgae on plant height(cm) of Finger millet at 30, 60 and 90 DAT

Treatment Details	Mean of three replicas		
	30 DAT	60 DAT	90 DAT
T_0	17.30	66.57	91.54
T_1	19.67	68.91	99.10
T_2	21.34	70.51	101.33
T_3	23.11[a]	72.67	103.16
T_4	23.89[a]	75.33	109.66
T_5	25.90	77.71	112.80
SEd(±)	0.55	0.60	0.52
CD(5%)	1.23	1.34	1.15

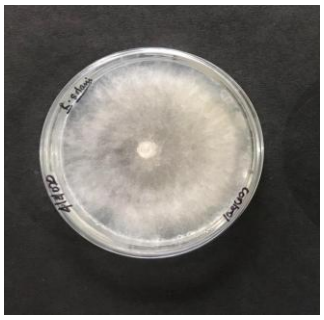
Means with similar letters represents non-significant to each other at 5% level
*Mean of Three Replications

Table.4 Effect of Microalgae on Yield parameters of Finger millet

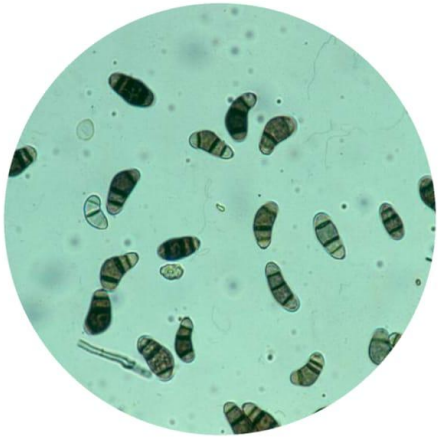
Treatment details	Yield components			Grain yield (q/ha)
	No. of Spikelets	Length of Spikelets	1000 grain weight	
T_0	4.67^{a}	7.30^{a}	2.60	19.32
T_1	5.33^{ab}	8.00_{ab}	2.82	21.70
T_2	5.67^{abc}	8.40^{b}	3.02	22.90
T_3	6.00^{abc}	9.47^{c}	3.23	23.50
T_4	6.33^{abc}	9.93^{c}	3.41	24.63
T_5	6.67^{bc}	11.57	3.65	25.42
SEd(±)	0.78	0.32	0.04	0.25
CD (5%)	1.75	0.72	0.09	0.57

Means with similar letters represents non-significant to each other at 5% level
*Mean of Three Replications.

Mycelium of *R.solani*Pure culture of *R.solani*



Conidia of *Curvularia* spp (under 40x)



Pure culture of *Curvularia* spp

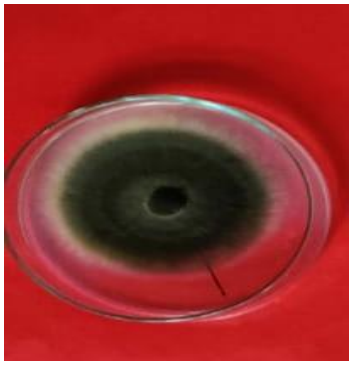


Plate.1 Radial growth (mm) of *Curvularia* spp.at 5% and 10 % concentration

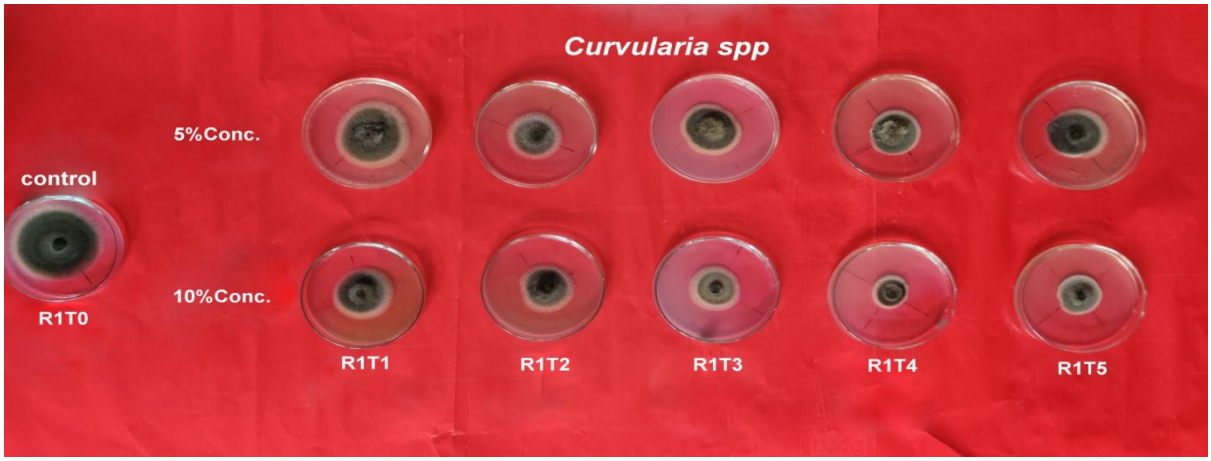


Fig.1 *In vitro* effect of botanicals on radial growth (mm) of *Curvularia* spp. at different concentrations

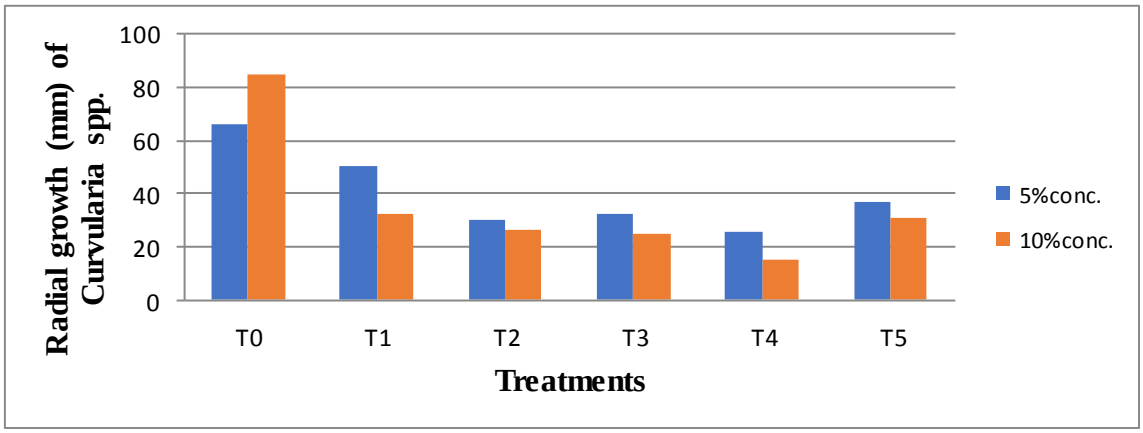


Fig.2 Effect of Microalgae on Disease incidence (%) at different DAI Sheath blight in fingermillet

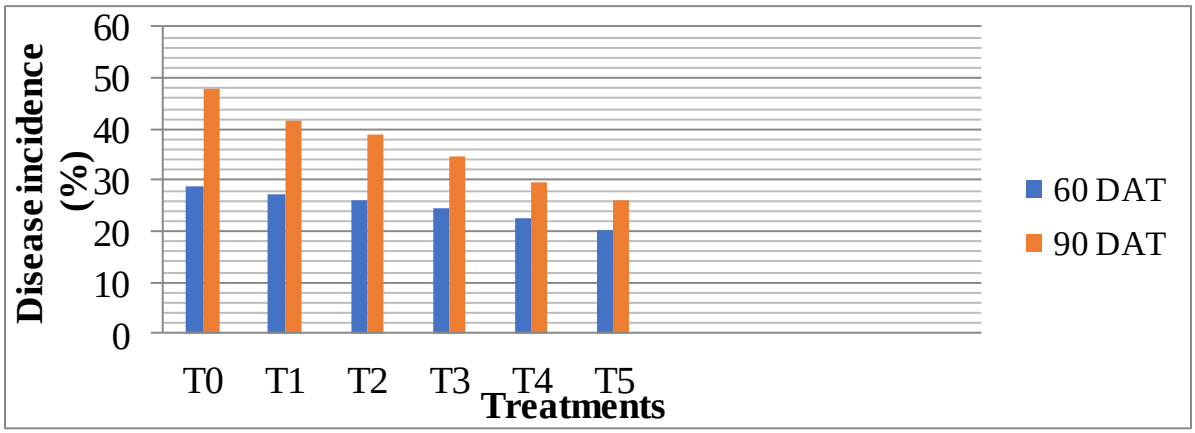


Fig.3 Effect of Microalgae on plant height (cm) of Finger millet at 30, 60 and 90 DAT

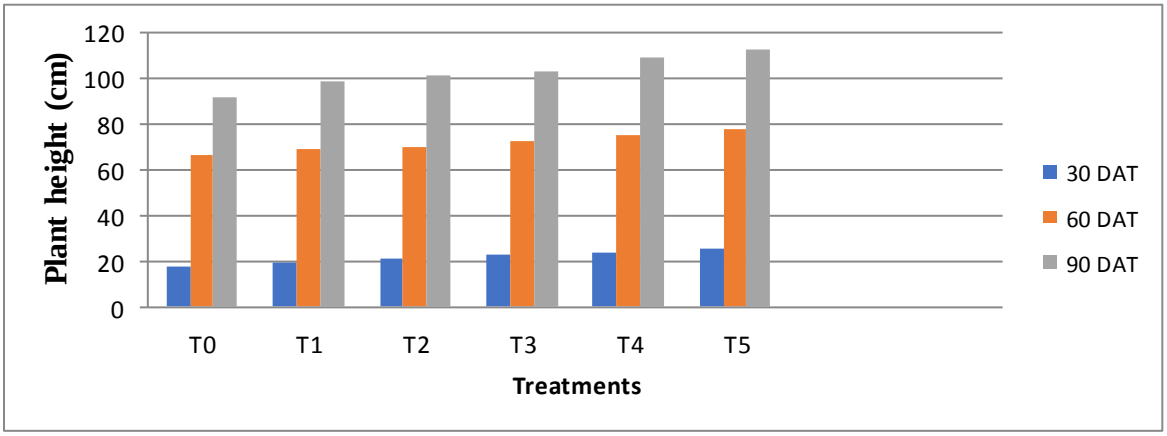
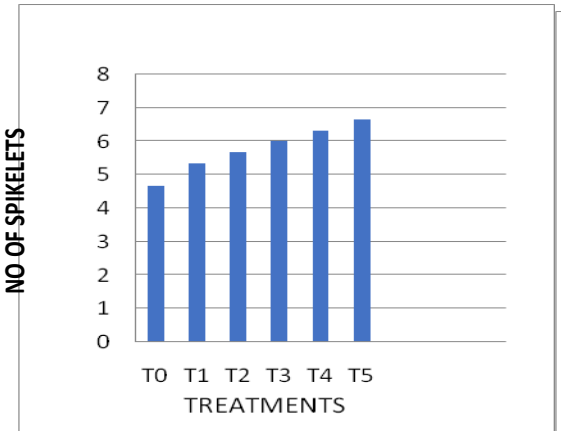
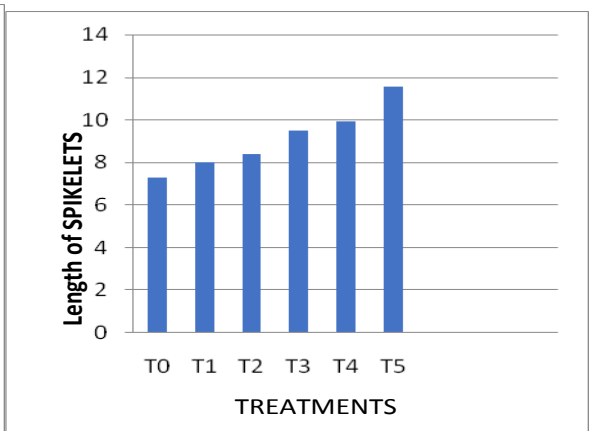
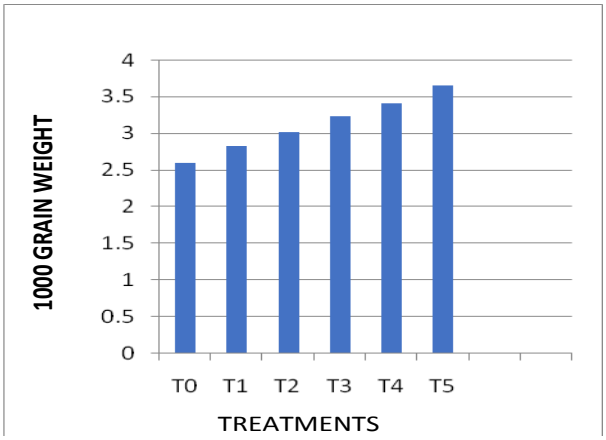
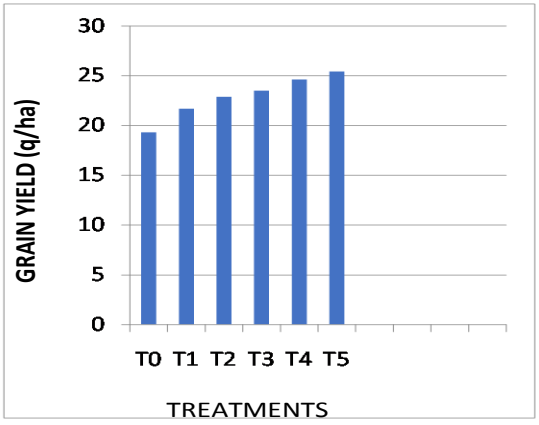


Fig.4 Effect of Microalgae on Yield parameters of Finger millet









However, the present study was limited to lab conditions in case of *Curvularia*, therefore to substantiate the present result more trials are needed for 2-3 seasons in field conditions for further recommendations.

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