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Original Article

FACTORS INFLUENCE ON GROWTH, DON AND NIV PRODUCTION BY TWO SPECIES OF *FUSARIUM* ISOLATED FROM FINGER MILLETS [*ELEUSINE CORACANA* L.]

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ABSTRACT

Objective: The present investigations (In vitro) on an influence of different synthetic and food based [flour] media, pH, temperature and microbial nutrients on growth, Deoxynivalenol [DON] and Nivalenol [NIV] production by *Fusarium aethiopicum* and *F. culmorum* was carried out.

Methods: *Fusarium* species associated with the finger millets were isolated and identified phenotypically and further confirmed by molecular methods by Polymerase Chain Reaction [PCR]. Monosporic seven day old *F. aethiopicum* and *F. culmorum* were grown in CYA broth and incubated at 27±2°C on rotary shaker for 21 days at 120 rpm. At the end of 21 day incubation period, cultures were harvested for determination of fungal biomass. The resultant culture filtrates were extracted twice with ethyl acetate and concentrated to get and final concentration of 1 ml in methanol and employed for RP-HPLC analysis for detection of DON and NIV production.

Results: Finger millets flour medium induced the highest amount of mycelial growth, DON and NIV production by *F. aethiopicum*. However, *F. culmorum* achieved highest amount of hyphal growth, DON and NIV production under the influence of yeast extract sucrose [YES] medium. Maize flour medium, rice flour medium and sorghum flour medium was next preffered substrates. Optimum pH of 5.5-6.5 and temperature of 20-35°C for growth, DON and NIV production was observed. Both the species of *Fusarium* failed to grow and produce toxins at pH 2.5-3.5 and temperature of 40°C. Yeast extract was most favorable for maximum DON and NIV production, which increased with an increase in its concentration. On the other hand, malt extract and beef extract induced good growth and mycotoxin production at comparatively higher concentration in both the species of *Fusarium* under study.

Conclusion: Comparatively food based media were the better substrates than synthetic media for both growth and DOIN and NIV production by two species of *Fusarium* under investigation. A positive correlation coefficient [r] on growth [0.458], DON [0.744] and NIV [0.882] was recorded among the media and both the species of *Fusarium*.

Keywords: Finger millets, *F. aethiopicum, F. culmorum*, Synthetic media, pH, Temperature, Microbial nutrients, DON, NIV, HPLC

INTRODUCTION

Finger millet [*Eleusine coracana* L.], commonly called as Ragi or African millet is grown primarily as a subsistence crop in parts of Sub-Saharan Africa and the Indian Sub-continent [1]. Finger millet is used as the staple food by tribal peoples, especially in dry areas of India [2]. It is rich in nutrients such as proteins, phosphorus, calcium, iron, amino acid and wholesome food for diabetics [3]. Finger millets are reported to be infested by different species of *Aspergillus, Penicillium* and *Fusarium* produce the wide range of mycotoxins which cause diverse health hazards to man and animals. *Fusarium* species that infest food and feed grains are reported to produce a wide range of mycotoxins [4]. Among all the cereal crops infested by *Fusarium* species, finger millets showed a high incidence [5].

Among different species of *Fusarium, F. aethiopicum* and *F. culmorum* which contaminate different food grains in pre- and/or post-harvest stages and produce Deoxynivalenol [DON] and Nivalenol [NIV] toxin chemotypes. Further, DON and NIV are known to be genotoxic, immuno-suppressive and teratogenic. Animals exposed to these toxins for long term results in organ failure. Among many natural substrates include maize, wheat and animal feed supporting DON and NIV production [6-8]. The natural occurrence of DON and NIV in finger millets has been reported from different parts of the world [9]. Mycotoxins produced by species of *Fusarium* may predispose livestock to infectious diseases, and this might in feed refusal and decreased productivity. Ingestion of mycotoxigenic foods by humans might contribute to decreased resistance to infectious agents and neoplasms, and these toxin chemo types may function as unrecognized etiological functions of immune disfunction diseases [10]. The capacity of DON to later normal immune functions is particularly important and evidence of DON can be immunosuppressive depending concentration and exposure of time it can be explained by the capacity of inhibiting protein synthesis [11].

Generally storage moulds grow in the temperature range of 10-40°C, pH 4–8 and water activity above 0.70 proliferate [12]. The substrate, moisture content and humidity in the storage atmosphere, available nutrients and co-existing indigenous fungi will favour the mycotoxigenic fungi and mycotoxins production [13]. In the present study influence of different synthetic and food based media, temperature, pH and microbial nutrients on growth and elaboration of DON and NIV production by *F. aethiopicum* and *F. culmorum* associated with finger millets was assessed and the results are discussed in this communication.

MATERIALS AND METHODS

Isolation and Identification of *Fusarium* species

Fusarium species associated with the finger millets were isolated by employing Blotter Technique [14]. The isolated *Fusarium* species were identified based on standard manuals and protocols available [15, 16]. The incidence of *Fusarium* species associated with finger millets in and around Telangana, India was reported our previous report [17]. The isolated species of *Fusarium* were maintained on Spezieller-Nahrstoffarmer Agar [SNA] slants. Based on cultural and morphological characters *F. aethiopicum* and *F. culmorum* were identified and were further confirmed by precise molecular methods by polymerase chain reaction [PCR]. The obtained sequences were submitted to National Center for Biotechnology Information [NCBI] with GenBank Accession number *F. aethiopicum* strain GSKUMB [KJ21085] and *F. culmorum* strain GSKUMB [KJ190159].

Influence of synthetic media on DON and NIV production

Nineteen following media were screened to find the suitable medium for growth; DON and NIV production by two species of *Fusarium* were studied.

1) Adye and Maltase medium [Glucose 50g; [NH_4] SO_4 3g; KH_2PO_4 10g; $MgSO_4$ $7H_2O$ 2g; Yeast extract 3g; Distilled water 1000 ml].

2) Asthana and Hawker medium A [Glucose 3g; KNO_3 3.5g; KH_2PO_4 1.75g; $MgSO_4$ $7H_2O$ 0.75g; Yeast extract 1g; Distilled water 1000 ml].

3) Czapek's medium [$NaNO_3$ 2g; KH_2PO_4 0.5g; KCl 0.5g; Yeast extract 7g; Sucrose 30g; Distilled water 1000 ml].

4) Glucose Asparagine medium [Glucose 20g; asparagines 5g; KH_2PO_4 3.4g; $MgSO_4.7H_2O$ 1.9g; NaCl 0.01g; Yeast extract 1g; Distilled water 1000 ml].

5) Glucose medium [Glucose 20g; Yeast extract 5g; Peptone 5g; NaCl 5g; K_2HPO_4 5g; distilled water 1000 ml].

6) Glucose Ammonium nitrate medium [Glucose 50g; NH_4 No_3 2.4g; KH_2PO_4 10g; $MgSo_4$ $7H_2O$ 2g; Yeast extract 1g; Distilled water 1000 ml].

7) Mineral liquid medium [Glucose 50g; NaNo3 2g; KCl 0.52g; $FeSO_4$ 0.01g; KH_2PO_4 1.52g; Yeast extract 1g; Distilled water 1000 ml].

8) Malt extract medium [Sucrose 30g; Malt extract [Difco] 40g; Peptone 1g; $CuSO_4.5H_2O$ 0.0005g; $ZnSO_4$ 0.001g; Yeast extract 1g; distilled water 1000 ml].

9) Richard's medium [KNO310g; KH2PO4 5g; MgSO4 7H2O 25g; Sucrose 35g; FeCl2 0.001g; Yeast extract 1g; Distilled water 1000 ml].

10) Semi-synthetic medium [Glucose 20g; NH4NO2 0.4g; KH2PO4 0.1g; KCl 0.3g; MgSO4 7H2O 0.04g; CaCl2 0.04g; CuSO4 0.1g; Sodium molybdate 0.1g; ZnSO4 0.1g; Yeast extract 1g; Distilled water 1000 ml].

11) SMKY medium [Sucrose 20g; $MgSO_4.7H_2O$ 0.5g; KNO_3 3g; Yeast extract 7g; Distilled water 1000 ml].

12) Singh and Wood medium [Glucose 25g; Asparagine 4g; KH_2PO_4 1g; $MgSO_4$ $7H_2O$ 0.75g; Peptone 10g; Yeast extract 1g; Distilled water 1000 ml].

13) Yeast extract sucrose medium [YES] [Yeast extract [Difco] 20g; Sucrose 15g; $MgSO_4$ $7H_2O$ 0.05g; $CuSO_4.5H_2O$ 0.0005g; $ZnSO_4$ 0.001g; Yeast extract 1g; Distilled water 1000 ml].

14) Rice flour medium: Rice flour 10g; 100 ml distilled water; heated to boil; filter, Yeast extract 10g; Distilled water 100 ml; pH 6.5.

15) Sorghum flour medium [Sorghum flour 10g; 100 ml distilled water; heated to boil; filter, Yeast extract 10g; Distilled water100 ml; pH 6.5].

16) Maize flour medium [Maize flour 10g; 100 ml distilled water; heated to boil; filter, Yeast extract 10g; Distilled water100 ml; pH 6.5].

17) Wheat flour medium [Wheat flour 10g; 100 ml distilled water; heated to boil; filter, Yeast extract 10g; Distilled water 100 ml; pH 6.5].

18) Bajra flour medium [Bajra flour 10g; 100 ml distilled water; heated to boil; filter; Yeast extract 10g; Distilled water100 ml; pH 6.5].

19) Finger millet flour medium [Finger millet flour 10g; 100 ml distilled water; heated to boil; filter, Yeast extract 10g; Distilled water 100 ml; pH 6.5].

Influence of pH

To study the influence of pH on growth and DON and NIV production by two species of *Fusarium* was studied by adjusting the pH of the medium [2.5, 3.5, 4.5, 6.5, 7.5, 8.5, 9.5 and 10.5] with the help of 6N HCl and/or 6 N NaOH. One ml [10^{-5}] spore suspension of *F. aethiopicum* and *F. culmorum* were aseptically inoculated and incubated at 27±2^{0}C on rotary shaker [Yihder LM-450 D] at 120 rpm for 21 days for growth and toxin production.

Table 1: Influence of Culture media on growth, DON and NIV production by two species of *Fusarium*

	F. aethiopicum			*F. culmorum*		
Name of the medium	**Dry. wt (mg/ml)**	**DON (µg/ml)**	**NIV (µg/ml)**	**Dry. wt (mg/ml)**	**DON (µg/ml)**	**NIV (µg/ml)**
Asthana & Hawkers medium	2.91±0.17	29.16±0.22	22.96±0.54	3.27±0.33	30.20±0.27	24.96±0.55
Singh & Wood medium	3.56±0.15	28.46±1.10	21.90±0.61	3.33±0.18	31.83±0.72	25.11±0.80
Malt extract medium	2.55±0.41	33.52±0.83	30.07±0.24	2.64±0.06	42.17±0.80	31.05±0.76
Glucose asparagine medium	2.61±0.45	28.18±0.64	20.77±0.48	3.07±0.08	45.78±0.56	29.20±0.59
YES Medium	3.00±0.28	48.20±1.26	32.38±0.61	3.14±0.25	52.99±0.74	41.95±0.55
Czapeck`s medium	2.25±0.17	43.27±0.81	31.51±0.99	3.37±0.25	52.10±0.43	36.69±0.22
SMKY medium	1.46±0.39	32.86±0.68	27.93±0.63	2.54±0.31	51.50±1.01	35.92±0.84
Glucose medium	3.38±0.26	34.11±0.30	25.78±0.43	3.53±0.32	32.72±0.07	30.08±0.44
Richards medium	2.84±0.22	31.84±0.82	21.93±0.40	3.21±0.23	36.36±0.19	21.40±0.18
Semi synthetic medium	2.49±0.18	30.93±0.67	28.03±0.60	2.53±0.34	38.63±0.29	30.21±0.53
Mineral liquid medium	2.37±0.25	21.70±0.57	19.76±0.32	2.35±0.23	24.77±0.52	20.12±0.71
Glucose-Ammonioum nitrate medium	3.32±0.18	34.09±0.60	25.81±0.41	2.97±0.35	34.58±0.37	30.11±0.70
Adye & Maltases medium	2.41±0.35	15.65±1.25	14.84±0.58	2.95±0.77	21.77±0.77	15.48±0.18
Rice flour medium	3.50±0.31	45.95±0.26	31.36±0.26	2.90±0.61	34.37±0.63	30.27±0.58
Maize flour medium	3.43±0.28	33.57±1.04	32.46±0.50	4.07±0.35	47.91±0.59	30.78±0.19
Sorghum flour medium	4.66±0.24	39.17±0.80	29.47±0.11	2.13±0.16	49.18±0.79	30.87±0.63
Wheat flour medium	2.76±0.15	32.66±0.23	25.98±0.29	4.27±0.06	38.85±0.65	27.46±1.75
Finger millet flour medium	6.14±0.13	52.85±0.69	32.46±0.18	4.44±0.21	52.91±0.47	33.36±0.29
Bajra flour medium	3.10±0.38	39.82±0.26	25.17±0.24	3.33±0.27	32.91±0.68	30.30±0.60

Influence of temperature

To find the suitable temperature for growth and DON and NIV production by two species of *Fusarium g*rowing cultures was incubated at different temperature [5, 10, 15, 20, 25, 30, 35, and 40°C] for 21 days. One ml [10^{-5}] spore suspension of *F. aethiopicum* and *F. culmorum* were added aseptically and incubated as described above.

Influence of microbial nutrients

To find the suitable microbial nutrients for growth and DON and NIV production by *F. aethiopicum* and *F. culmorum* by employing different microbial nutrients [Yeast extract, Beef extract, Peptone and Malt extract] at two concentrations [0.5 and 1.0%] aseptically just before inoculation. One ml spore suspension [10^{-5}] of *F. aethiopicum* and *F. culmorum* were added aseptically and incubated as described above.

Determination of biomass of *Fusarium* species

At the end of 21 d incubation period, cultures of *Fusarium* species were harvested on pre-weighed Whatmann No.42 filter paper. The filter paper along with mycelial mat was dried in a hot air oven at 65-75°C for 72 hrs to obtain constant weight in an analytical balance after cooling to room temperature in a desiccator and biomass yield per ml of medium was calculated.

Extraction and cleanup of DON and NIV

Seven days old monosporic culture of *F. aethiopicum* and *F. culmorum* were grown individually in 250 ml of Erlenmeyer conical flask containing 100 ml SNB broth for 5 days and harvested on Whatman 42 filter paper and filtered the mycelium and spore suspension [10^{-5}] was transferred to CYA broth aseptically and

incubated at 27±2°C on rotary shaker [Yiedher LM-450D] for 21 days at 120 rpm. At the end of incubation period, cultures were harvested on Whatman filter paper No.42 and resultant culture filtrates were centrifuged at 12,000g to get cell-free filtrates. The resultant culture filtrates were acidified with 0.1M *o*-phosphoric acid and extracted twice with ethyl acetate [1:1, v/v] and concentrated by rotary evaporator to get and final concentration of 1 ml in methanol and employed for HPLC analysis.

Table 2: Statistical analysis of media, pH, temperature and microbial nutrients on growth, DON and NIV production by two species of *Fusarium*

	Dry. wt (mg/ml)		DON (µg/ml)		NIV (µg/ml)	
	F. aethiopicum	*F. culmorum*	*F. aethiopicum*	*F. culmorum*	*F. aethiopicum*	*F. culmorum*
Media						
Minimum	1.443	2.137	15.66	16.12	11.42	15.11
Maximum	6.143	4.440	52.85	52.99	32.46	41.95
Mean	3.013	3.127	33.59	38.39	25.60	28.53
Std. Dev	1.037	0.6214	9.646	10.82	5.908	6.702
Std. Error	0.2318	0.1390	2.157	2.420	1.321	1.499
t, df	13.00 /19	22.51 /19	15.57 /19	15.86 /19	19.38/19	19.03/19
Correlation	0.458/0.042	---	0.744/0.0002	---	0.882/0.0001	---
pH						
Minimum	0.0	0.0	0.0	0.0	0.0	0.0
Maximum	5.367	6.367	98.54	93.66	95.58	88.70
Mean	3.074	4.038	44.59	46.30	41.33	41.79
Std. Dev	1.969	2.355	42.28	36.64	37.69	35.21
Std. Error	0.6563	0.7848	14.09	12.21	12.56	11.74
t, df	4.684 /8	5.145/8	3.164/8	3.790/8	3.290/8	3.561/8
Correlation	0.809/0.008	---	0.962/0.00003	---	0.991/0.0001	---
Temperature						
Minimum	0.0	0.0	0.0	0.0	0.0	0.0
Maximum	6.467	7.203	64.88	66.12	43.95	64.21
Mean	4.277	4.565	37.02	35.32	22.59	32.58
Std. Dev.	2.582	2.703	25.84	26.06	17.08	23.93
Std. Error	1.155	1.209	11.56	11.65	7.636	10.70
t, df	3.705 df=4	3.776/4	3.203/4	3.031/4	2.958/4	3.044/4
Correlation	0.977/0.004	---	0.904/0.035	---	0.994/0.0005	---
Microbial nutrients						
Minimum	3.6	4.367	24.4	20.88	19.03	16.54
Maximum	5.7	6.267	52.3	50.87	42.47	40.69
Mean	4.988	5.523	36.9	31.57	28.67	28.53
Std. Dev.	0.7689	0.6481	10.1	9.564	7.109	7.882
Std. Error	0.2563	0.216	3.38	3.188	2.37	2.627
t, df	19.46/8	25.56/8	10.92/8	9.902/8	12.10/8	10.86/8
Correlation	0.655/0.055	---	0.860/0.003	---	0.728/0.026	---

Analysis and Quantification of DON and NIV by HPLC

Liquid chromatography [LC] analysis of DON and NIV was carried out by using *JASCO-975*[Japan], C-18 isocratic reverse phase column [250X4.6 mm internal diameter, 5µM particle size] by injecting 20 µl of sample extract. The mobile phase for toxin consists of a mixture of methanol: water [70:30] [Sigma Aldrich, Mumbai, India]. The pH was adjusted, degassed using bath sonicator [PCI™ Analytics]. The chromatography was performed isocritically at a flow rate of 0.5 ml/min at a wavelength of 227 nm under UV detector. The amount of DON and NIV produced was determined by HPLC fluorometric response compared with standard DON and NIV [Sigma Aldrich, Mumbai, India].

Statistical Analysis

Statistical analysis of significance difference of the data tested by one sample t test and coefficient of variation were applied to compare the growth, DON and NIV production similarities [P< 0.0005] using GraphPad InStat version 5.03 [GraphPad Software, Inc.,]

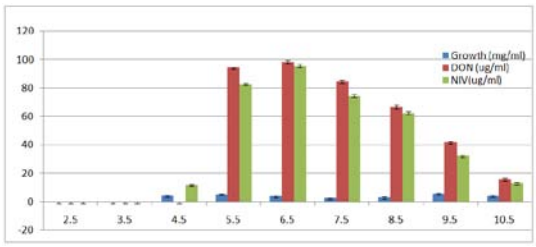

Fig. 1: Influence of pH on growth, DON and NIV production by *F. aethiopicum*

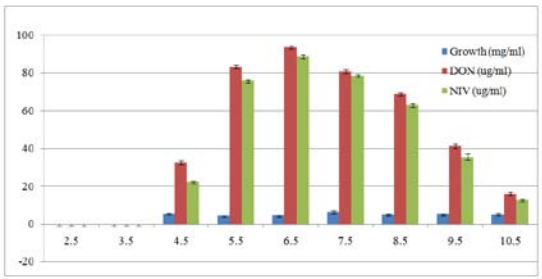

Fig. 2: Influence of pH on growth,DON and NIV production by *F. culmorum*

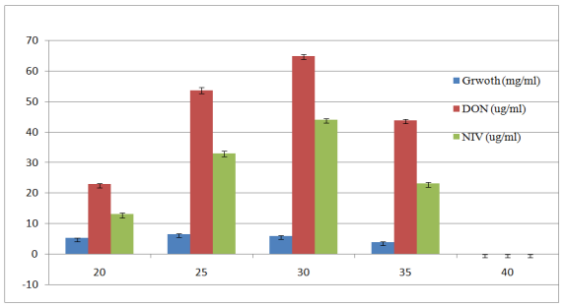

Fig. 3: Influence of temperature on growth, DON and NIV production by *F. aethiopicum*

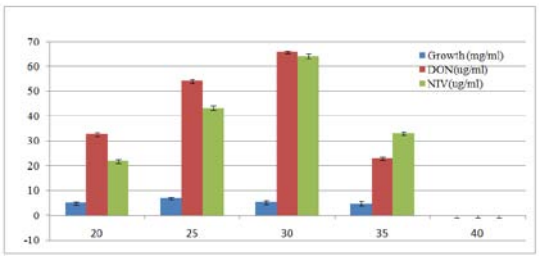

Fig. 4: Influence of temperature on growth, DON and NIV production by *F. culmorum*

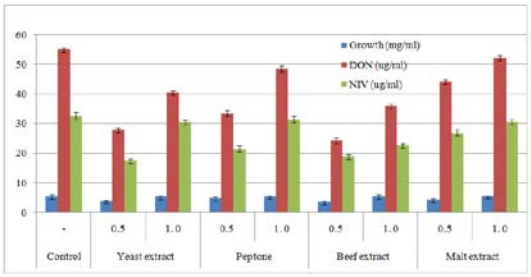

Fig. 5: Influence of microbial nutrients on growth, DON and NIV production by *F. aethiopicum*

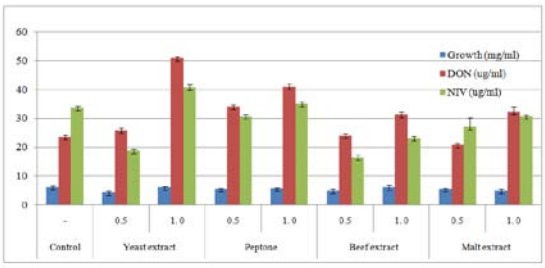

Fig. 6: Influence of microbial nutrients on growth, DON and NIV production by *F. culmorum*

RESULTS

Influence of media on growth, DON and NIV production

The present investigations on mycelial growth, DON and NIV production by *F. aethiopicum* and *F. culmorum* raveled that DON and NIV production by *F. aethiopicum* was maximum in finger millets medium followed by YES, maize flour, rice flour, sorghum flour and Czapek's medium in a descending order [Table 1]. Adye and Maltases, Mineral Liquid medium, Glucose-Asparagine, SKMY and Singh and Wood medium were poor substrates for both toxins production. Rest of the media supported intermediate amount of DON and NIV production. However, *F. culmorum* apted YES medium followed by Finger millets flour medium, Czapek's, SKMY and Sorghum flour medium for production of DON and NIV.

The DON and NIV production was least in Adye and Maltase, Mineral Liquid and Singh and Wood medium in a descending order. Rest of the media responsible for intermediate amount of toxin productions. *F. aethiopicum* and *F. culmorum* achieved maximum biomass in finger millets medium followed by sorghum flour, rice flour and glucose medium in a descending order. Mineral liquid, Semi-synthetic, Czapeck's and Glucose-asparagine medium were responsible for the lowest amount of biomass production. Rest of the media were responsible for moderate mycelium yield of both the species of *Fusarium* under study.

Statistical analysis of influence of different media on growth, DON and NIV production by *F. aethiopicum* and *F. culmorum* was carried out and their minimum, mean, maximum and standard deviations were depicted in table 2. The mean growth of *F. aethiopicum* 3.01mg/ml, DON 33.59µg/ml, and NIV 25.6 µg/ml production was recorded under influence of different medium. On the other hand, *F. culmorum* supported 3.12mg/ml, DON 38.39 µg/ml and NIV 28.53µg/ml. A positive correlation coefficient [r] was recorded between the growth [0.458], DON [0.744] and NIV [0.882] production by *F. aethiopicum* and *F. culmorum*

Influence of pH on growth, DON and NIV production

Influence of pH on growth and DON and NIV production by two species of *Fusarium* revealed that, *F. aethiopicum* and *F. culmorum* accomplished good growth at pH 6.5, and failed to grow at pH 2.5 and 3.5 by both the species of *Fusarium* under study. Increase of acidity or alkalinity resulted in gradual decrease in the biomass, DON and NIV production by *F. aethiopicum* [Fig 1] and *F. culmorum* [Fig 2]. On the other hand, pH 5.5 and 6.5 were optimum and decreased with further increase of acidity or alkalinity. Interestingly, trace amount of DON, NIV elaboration could be recorded at pH 10.5 by both the present species of *Fusarium*.

Different pH influenced the mean growth of *F. aethiopicum* 3.074mg/ml, DON 44.59µg/ml and NIV 41.33µg/ml was recorded. However, mean growth of *F. culmorum* 4.03mg/ml, DON 46.30µg/ml and NIV 41.79µg/ml was recorded significantly. A positive correlation between growth [0.809], DON [0.962] and NIV [0.991] production was recorded by *F. aethiopicum* and *F. culmorum*.

Influence of temperature on growth, DON and NIV production

The temporal studies showed highest amount of biomass, DON and NIV production was recorded at 30°C by both the species of *Fusarium* and they failed to grow and produce toxin at 40°C. *F. aethiopicum* and *F. culmorum* could grow over a wide range of temperature [20-35°C] with a maximum growth at 30°C. The marginal growth and DON and NIV production was recorded in the temperature range of 20-35°C, optimum at 30°C for both the species [Fig3&4].

Statistical analysis of influence of incubation temperature revealed the mean growth 4.27mg/ml, DON 37.02µg/ml, and NIV 22.59µg/ml was recorded with *F. aethiopicum.* In contrary, *F. culmorum* was recorded mean growth 4.565 mg/ml, DON 35.32µg/ml] and NIV 32.58µg/ml significantly. A correlation coefficient [r] on biomass [0.655], DON [0.860] and NIV [0.728] was recorded between the *F. aethiopicum* and *F. culmorum* among the different temperature tried.

Influence of microbial nutrients on growth, DON and NIV production

In order to find the suitable microbial nutrients for growth, DON and NIV production different microbial nutrients were assessed. Most of the microbial nutrients added to the medium stimulated growth and DON and NIV production by *F. aethiopicum* and *F. culmorum*. The degree of stimulation increased with the increase in the concentration of microbial nutrients. Yeast extract was responsible for production of good amount of DON and NIV by *F. aethiopicum* [Fig 5] and *F. culmorum* [Fig 6] which increased with the increase in their concentration. Malt extracts and Beef extract could stimulate DON and NIV production only at higher concentration. Rest of microbial nutrients supported marginal increase in DON and NIV production.

Influence of microbial nutrients showed mean growth of *F. aethiopicum* 4.98mg/ml, DON 36.9µg/ml, ranged from 24.4 and 52.3µg/ml and mean NIV 28.67µg/ml, ranged 19.03 to 42.47µg/ml produced by *F. aethiopicum.* On the other hand, *F. culmorum* produced mean biomass 5.52mg/ml, DON 31.57µg/ml, NIV 38.39 µg/ml was recorded significantly. A correlation coefficient [r] on biomass [0.655], DON [0.860] and NIV [0.728] was recorded between the *F. aethiopicum* and *F. culmorum* among the microbial nutrients tried.

DISCUSSION

A large number of moulds associated with different agricultural commodities both in the field and storage and elaborate mycotoxins

which are biologically active are known to cause serious health hazards to human and animals. Finger millets were frequently infested by *Fusarium* species and get contaminated by wide range of mycotoxins. Infestations of finger millets by species of *Fusarium* increased with congenial environmental factors such as moisture, nutritional composition of the millet and the ambient temperature and elaborate variety of mycotoxins [18].

Present study aimed to find out the conditions favorable for growth, DON and NIV production by species of *Fusarium*. Our observation reveal that finger millet medium was the ideal substrate for the growth and DON and NIV which is in agreement with the observation of Narasimha Rao et al. [19] who reported Czapeck's medium and YES medium to be the best substrates for the growth and fumonisins [B1] production by *Fusarium moniliforme*. Koteswara Rao et al. [20] have also reported Czepak Yeast Autolysate [CYA] medium stimulated OTA production by *Penicillium verrucosum* and *P. nordicum*. They further stated that food based media [supplemented with 1% yeast extract] to be the best substrates for toxin production than synthetic media. The DON and NIV production by *F. aethiopicum* and *F. culmorum* were quite similar but differed in dynamics of its production which may be attributed to genetic makeup of the species. The present investigation reveals that the substrate influenced almost to the same degree on biomass yield and DON, NIV production.

In the present investigations *F. aethiopicum* and *F. culmorum* were grown at different pH, but varied in the amount of biomass and toxin production. These species were observed it can able to grown in strong acidic [pH] as well as high alkaline condition. The results obtained in the present study are positively correlated with Wheeler *et al.* [21] who also reported that of pH was ideal for growth and mycotoxin production by *Aspergillus, Penicillium* and *Fusarium* species. From the present investigations, it is clear that pH plays major role on DON and NIV production.

Temporal studies have shown that *Fusarium* species failed to grow below 20°C and above 35°C. The increase or decrease of incubation temperature inhibited both DON and NIV production as with the growth of the fungus. Surekha and Reddy [22] have also reported definite influence of temperature and pH on the production of penitrem B by *P. aurantiogriseum.* Mycotoxin production by two species of *Fusarium* under study was totally inhibited at 40°C. Low and high temperatures adversely affected DON and NIV production by both the present species of *Fusarium.* However, the temperature [25°C] used now appears to be suboptimal for OTA production by *Fusarium* species. In the present study, the production of DON and NIV production over a range of environmental conditions are important as it could assess in predicting the possible risk of DON and NIV production. The present study also demonstrated temperature 30°C optimum for DON and NIV production are in agreement with Mitchell et al. [23] also demonstrated relations between water activity and temperature on growth and OTA production by *Aspergillus carbonarius* strains.

In contrast 1% yeast extract added to the medium stimulated the DON and NIV production, which further increased with the increase in its concentration. Malt extracts also responsible for stimulation of DON and NIV production comparatively at higher concentration. Previous studies by Cabanes et al. [24] reported that *A. carbonarius* isolates produced maximum OTA on unmodified yeast extract sucrose agar and Czapek yeast agar after about 14 days although some were detected after 7 days with a subsequent decrease over time. Influence of microbial nutrient varied both with species are agreement with Narsimha Rao et al. [19]. Koteswara Rao et al. [20] have also recorded influence of varied microbial nutrients on growth and OTA production by *Penicillium* species.

CONCLUSION

The conclusions of the present investigations, food based media accomplished good growth and DON and NIV production by both the species of *Fusarium* as compared with synthetic medium and also influence of different a biotic condition viz., pH, temperature and microbial nutrients are also major important factors in predicting the possible risk of DON and NIV production by *F. aethiopicum* and *F. culmorum* infestation in finger millet and some small grain crops.

CONFLICT OF INTERESTS

We declare that we have no conflict of interest.

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ABBREVIATIONS

DON: Deoxynivalenol

NIV: Nivalenol

PCR: Polymerase Chain Reaction

CYA: Czepak Yeast Autolysate Agar

SNA: Spezieller-Nahrstoffarmer Agar

YES: Yeast Extract Sucrose Medium

RP-HPLC: Reverse Phase High Performance Liquid Chromatography.

HPLC: High Performance Liquid Chromatography.

National Center for Biotechnology Information [NCBI]

HCl: Hydrochloric acid

NaOH; Sodium hydroxide

UV: Ultraviolet

OTA: Ochratoxin A

r: correlation coefficient

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