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Vol. 19(3)

September-December, 2021

CONTENTS

Unrevealing the role of epistasis through Triple Test Cross in Indian mustard NARENDER SINGH, USHA PANT, NEHA DAHIYA, SHARAD PANDEY, A. K. PANDEY and SAMEER CHATURVEDI	330
Testing of InfoCrop model to optimize farm resources for mustard crop under <i>tarai</i> region of Uttarakhand MANISHA TAMTA, RAVI KIRAN, ANIL SHUKLA, A. S. NAIN and RAJEEV RANJAN	335
<i>In vitro</i> evaluation of endophytes and consortium for their plant growth promoting activities on rice seeds DAS, J., DEVI, R.K.T. and BARUAH, J.J.	342
Effect of subsurface placement of vermicompost manure on growth and yield of wheat (<i>Triticum aestivum</i> L. Var. UP 2526) ABHISHEK KUMAR and JAYANT SINGH	348
Assessment of different nutrient management approaches for grain yield, gluten content and net income of common bread wheat (<i>Triticum aestivum</i> L.) in Western Himalayan region of Uttarakhand BHAWANA RANA and HIMANSHU VERMA	359
Suitability assessment of land resources for cassava (<i>Manihot esculenta</i> L.) and yam (<i>Dioscorea spp</i> L.) cultivation in Khana LGA, Rivers State, Southern Nigeria PETER, K.D., UMWENI, A.S. and BAKARE, A.O.	367
Biophysical and biochemical characters conferring resistance against pod borers in pigeonpea PARUL DOBHAL, R. P. MAURYA, PARUL SUYAL and S.K. VERMA	375
Population dynamics of major insect pest fauna and their natural enemies in Soybean SUDHA MATHPAL, NEETA GAUR, RASHMI JOSHI and KAMAL KISHOR	385
Fumigant toxicity of some essential oils and their combinations against <i>Rhizopertha dominica</i> (Fabricius) and <i>Sitophilus oryzae</i> (Linnaeus) NIDHI TEWARI and S. N. TIWARI	389
Long term efficacy of some essential oils against <i>Rhizopertha dominica</i> (Fabricius) and <i>Sitophilus oryzae</i> (Linnaeus) NIDHI TEWARI and S. N. TIWARI	400
Management strategies under chemicals, liquid organic amendments and plant extracts against black scurf of potato caused by <i>Rhizoctonia solani</i> Kühn in <i>tarai</i> regions of Uttarakhand SURAJ ADHIKARI, SHAILBALA SHARMA, R. P. SINGH, SUNITA T. PANDEY and VIVEK SINGH	408
Effective management strategies against ginger rhizome rot caused by <i>Fusarium solani</i> by the application of chemicals, bioagents and Herbal <i>Kunapajala</i> in mid hills of Uttarakhand SONAM BHATT, LAXMI RAWAT and T. S. BISHT	417

Distribution and morphological characterisation of isolates of <i>Fusarium moniliforme</i> fsp. <i>subglutinans</i> causing Pokkah Boeng disease of sugarcane in different sugarcane growing areas of Udham Singh Nagar district of Uttarakhand HINA KAUSAR, BHAGYASHREE BHATT and GEETA SHARMA	429
Biointensive management of <i>Meloidogyne enterolobii</i> in tomato under glasshouse conditions SHUBHAM KUMAR, ROOPALI SHARMA, SATYA KUMAR and BHUPESH CHANDRA KABDWAL	435
Effect of pre-harvest application of eco-friendly chemicals and fruit bagging on yield and fruit quality of mango KIRAN KOTHIYAL, A. K. SINGH, K. P. SINGH and PRATIBHA	447
A valid and reliable nutrition knowledge questionnaire: an aid to assess the nutrition friendliness of schools of Dehradun, Uttarakhand EKTA BELWAL, ARCHANA KUSHWAHA, SARITA SRIVASTAVA, C.S. CHOPRA and ANIL KUMAR SHUKLA	452
Potential of common leaves of India as a source of Leaf Protein Concentrate RUSHDA ANAM MALIK, SHAYANI BOSE, ANURADHA DUTTA, DEEPA JOSHI, NIVEDITA, N.C. SHAHI, RAMAN MANOHARLAL and G.V.S. SAIPRASAD	460
Job strain and muscle fatigue in small scale unorganized agri enterprises DEEPA VINAY, SEEMA KWATRA, SUNEETA SHARMA and KANCHAN SHILLA	466
Drudgery reduction of farm women involved in weeding of soybean crop SHALINI CHAKRABORTY	475
Childhood obesity and its association with hypertension among school-going children of Dehradun, Uttarakhand EKTA BELWAL, K. UMA DEVI and APARNA KUNA	482
Spring water and its quality assessment for drinking purpose: A review SURABHI CHAND, H.J. PRASAD and JYOTHI PRASAD	489
Spatial distribution of water quality for Indo-Gangetic alluvial plain using Q-GIS SONALI KUMARA, VINOD KUMAR and ARVIND SINGH TOMAR	497
Application of geospatial techniques in morphometric analysis of sub-watersheds of Nanak Sagar Catchment AISHWARYA AWARI, DHEERAJ KUMAR, PANKAJ KUMAR, R. P. SINGH and YOGENDRA KUMAR	505
Evaluation of selected carbon sources in biofloc production and carps growth performance HAZIQ QAYOOM LONE, ASHUTOSH MISHRA, HEMA TEWARI, R.N. RAM and N.N. PANDEY	516
Calcium phosphate nanoparticles: a potential vaccine adjuvant YASHPAL SINGH and MUMTESH KUMAR SAXENA	523
Factors affecting some economic traits in Sahiwal Cattle DEVESH SINGH, C. B. SINGH, SHIVE KUMAR, B.N. SHAHI, BALVIR SINGH KHADDA, S. B. BHARDWAJ and SHIWANSHU TIWARI	528
The effect of probiotics and growth stimulants on growth performance of Murrah Buffalo SAMEER PANDEY, RAJ KUMAR, D.S. SAHU, SHIWANSHU TIWARI, PAWAN KUMAR, ATUL SHARMA and KARTIK TOMAR	532

Distribution and morphological characterisation of isolates of *Fusarium moniliforme* fsp. *subglutinans* causing Pokkah Boeng disease of sugarcane in different sugarcane growing areas of Udham Singh Nagar district of Uttarakhand

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ABSTRACT: Pokkah Boeng disease is one of the most important diseases of sugarcane and is coming up as a serious issue of concern for sugarcane growers. *Fusarium moniliformae* variability persisting in different areas may have correlation with the severity of the disease prevalent in those areas. Therefore, a study was conducted to find out morphological variability of the isolates of pathogen in different sugarcane growing areas in Udham Singh Nagar district of Uttarakhand. Twenty isolates of the *Fusarium moniliformae* from Kichha, Sitarganj, Khatima, Nanakmatta, Bazpur, Pantnagar, Jawahar nagar and Kashipur showed variation in colony characteristics, conidial shape and size, indicating the existence of variability among them. Colony texture of the isolates varied from fluffy profuse submerged to cottony and wide range of pigmentation pattern was observed. Macro conidia in different isolates ranged from 21.8- 8.74µm.

Key words: Morphology, Pokkah Boeng, pigmentation

Sugarcane (*Saccharum officinarum* L.) also known as Ganna/Eekh is the most important sugar crop, contributing about more than 70 per cent of the world sugar production (Gawade *et al.*, 2012). It is a vegetatively propagated crop, grown in more than 80 countries across the world and produces 11.8 MT of sugar (Ali, 2015). India is the second largest producer of sugarcane next to Brazil and the world's leading sugar consumer. Pokkah boeng disease caused by *Fusarium moniliformae* is one of the most important fungal diseases of sugarcane. Earlier the disease was of little concern but recently it is emerging as a serious disease due change in climate patterns and increased area under early maturing varieties of sugarcane. The disease was first reported from Java (Indonesia) in 1896 by Walker and Went and thereafter studied by several workers (Martin *et al.*, 1961). The disease is caused by the pathogen *Fusarium moniliformae* was firstly described by Sheldon (1904). The perfect stage of the pathogen is *Gibberella fujikuroi* (Sawada). From Malaysia, the disease was recorded in 1973 and it was concluded that the disease occurs when crops were grown in a climatic condition where hot and dry season

followed by a wet season persists which was helpful for the spread of the pathogen causing Pokkah boeng disease (Siti *et al.*, 2008).

Pokkah boeng disease of sugarcane has been reported from all sugarcane growing regions with different disease severities (Viswanathan, 2012; Vishwakarma *et al.*, 2013). In India, the disease was first time reported from Maharashtra in 1983-84 on two commercial varieties CoC671 and Co7219, and was a major constraint for sugarcane production (Patil and Hapase, 1987). Ricaud *et al.* (2012) reported that this disease causes 10-38 per cent yield losses in the susceptible variety POJ2878. Reduction in cane yield parameters like cane weight, length of internodes, total juice, girth and sugar per cent was also observed (Singh *et al.*, 2006). The sugar production losses may range upto 40.8-64.5 per cent (Duttamajumder, 2004). The disease symptoms of Pokkah boeng has been observed to occur in four phase's namely Chlorotic phase I, Chlorotic phase II, Knife cut phase and Acute/Top-rot. The earliest symptoms include the development of Chlorotic patches towards the base of young leaves. The affected leaves are distorted and shortened, appearance



Fig 1: Symptoms produced by *Fusarium moniliforme* f sp. *subglutinans* in field; A. Chlorosis stage, B. Leaf twisting stage, C. Knife cut stage, D. Top Rot stage

of lesions on the stalk, top-rot occurs in the young spindle and the entire top of the plant dies.

Several surveys have been conducted by many researchers and the disease was observed in almost all the sugarcane growing area with more or less disease incidence. Singh *et al.* (2006) conducted a survey during monsoon and post monsoon season in central Uttar Pradesh and observed that disease incidence varied from 0.1 to 10.0 per cent. Vishwakarma *et al.* (2013) conducted a survey

during 2007-2013 in Uttar Pradesh and found increase in the trends of the disease. Sharma *et al.* (2014) conducted a field survey during 2011-12 in major sugarcane growing districts of Uttar Pradesh and observed 1.4-30 per cent incidences. Looking into the serious losses caused by the disease and the increasing incidence of the disease, a study was conducted to observe whether with the difference in disease incidence in different regions, is there some variability among the isolates of *Fusarium moniliformae* collected from different

Table 1: List of isolates of *Fusarium moniliformae* collected from different locations

S.No.	Isolates/ Code	Variety	Location	Year
1.	FmU1	Co89003	Kiccha sugarmill compound	2017
2.	FmU2	Co0238	Kiccha	2017
3.	FmU3	CoPant3220	Kichcha	2017
4.	FmU4	Co118	Kiccha sugarmill compound	2017
5.	FmU5	Co118	Kiccha	2017
6.	FmU6	Co118	Kanchanpuri, Khatima	2017
7.	FmU7	Co0238	Kanchanpuri, Khatima	2017
8.	FmU8	Co99214	Kanchanpuri, Khatima	2017
9.	FmU9	CoH160	Jamor, Sitarganj	2017
10.	FmU10	Co0238	Jamor, Sitarganj	2017
11.	FmU11	Co118	Jamor, Sitarganj	2017
12.	FmU12	Co0239	Jamor, Sitarganj	2017
13.	FmU13	Co167	Keshawala, Bazpur	2017
14.	FmU14	Co88239	Mariyampur, Bazpur	2017
15.	FmU15	Co0238	Jaitpur, Kashipur	2017
16.	FmU16	Co118	Judka, Kashipur	2017
17.	FmU17	Co88230	Jawaharnagar, Pantnagar	2017
18.	FmU18	CoPant 3220	Pantnagar	2017
19.	FmU19	CoPant 5224	Pantnagar	2016
20.	FmU20	Co0238	Pantnagar	2016

regions.

MATERIALS AND METHODS

The present study investigated the occurrence of Pokkah Boeng disease of sugarcane in district Udham Singh Nagar in order to provide scientific reference for sugarcane variety distribution and disease occurrence. A survey was conducted during monsoon season in the month of July-August in major sugarcane growing areas of district Udham Singh Nagar namely Kichha, Sitarganj, Khatima, Nanakmatta, Bazpur, Pantnagar, Jawaharnagar and Kashipur. The areas under sugarcane cultivation were visited with the help of sugarcane supervisors, sugar mill employees, sugarcane corporative committee members and farmers. The first field visit was

made in Kichha Jawahar Nagar regions, followed by Sitarganj, Khatima, Nanak Matta, Bazpur and Kashipur in the month of July. Observations were taken cultivar wise and disease infection was recorded.

Cultural and Morphological Variability of Pathogen

The 20 isolates collected from different location of district were designated as FmU1-FmU20, were studied for their variability test on the basis of cultural and morphological variability. Morphological studies of the isolates were conducted to find out the variability among the isolates by comparing their conidial population, shape, size colour and septation of the conidia. A small amount of pure culture of the isolates was taken from four position of 7 days old culture

Isolate No.	Colour of colony	Characters of colony	Growth Pattern	Macro-conidia (size in μm)	Micro-conidia (size in μm)	Colony (dia. in mm) at 10 th days
FmU1	Peach yellow	Mycelium cottony white to submerged	luxuriant	15.54-16.5	5.24-6.32	71
FmU2	Light orange	Mycelium fluffy to partially submerged	scarce	21.8-26.4	8.68-12.3	90
FmU3	Salmon pink		scarce	12.14-14.2	5.58-6.2	85
FmU4	White	Cottony floccose mycelium	luxuriant	8.7-10.8	5.1-6.1	76
FmU5	Whitish cream	Fluffy growth with light buff	fluffy growth to pale cream	8.74-11.23	3.42-4.85	68
FmU6	Light yellow	Aerial mycelial, thread like hyphae	Moderate	15.24-17.32	5.28-6.28	75
FmU7	Dark maroon	Mycelium fluffy to partially submerged	abundant	14.08-16.31	7.56-8.25	71
FmU8	Whitish cream	Thread like mycelium at centre	luxuriant	10.63-11.4	4.4-5.1	80
FmU9	Peach colour	Cottony coarse growth	abundant	10.38-12.2	3.5-5.77	76
FmU10	Light yellow	Fast growing mycelium feathery growth	luxuriant	13.35-14.4	4.38-5.828	90
FmU11	Violet	cottony growth	luxuriant	10.38-11.34	5.1-6.25	81
FmU12	Cream yellow	Thread like light mycelium growth	scarce	13.6-16.7	5.3-7.3	90
FmU13	White	Fluffy white mycelium	luxuriant	9.71-11.8	6.5-7.2	76
FmU14	Dark yellow	Cottony floccose growth		16.94-18.9	7.5-8.8	78
FmU15	Dark brown	Whitish pale colour of colony cottony growth	Moderate	16.5-18.3	7.285-9.01	82
FmU16	Light yellow	Pinkish colour of colony	scarce	12.5-13.4	6.7-7.2	75
FmU17	Salmon pink	Dense cottony colony at centre	luxuriant	11.84-13.4	6.5-6.9	90
FmU18	Violet	Whitish cream mycelial growth, fast growing growth pattern	abundant	11.6-13.4	6.01-7	90
FmU19	White	White fast growing fluffy mycelial colony	luxuriant	11.6-12.3	5.43-6.74	73
FmU20	Purple	Fast growing white mycelial colony later turn into purple colour	luxuriant	9.47-10.65	4.3-5.58	87

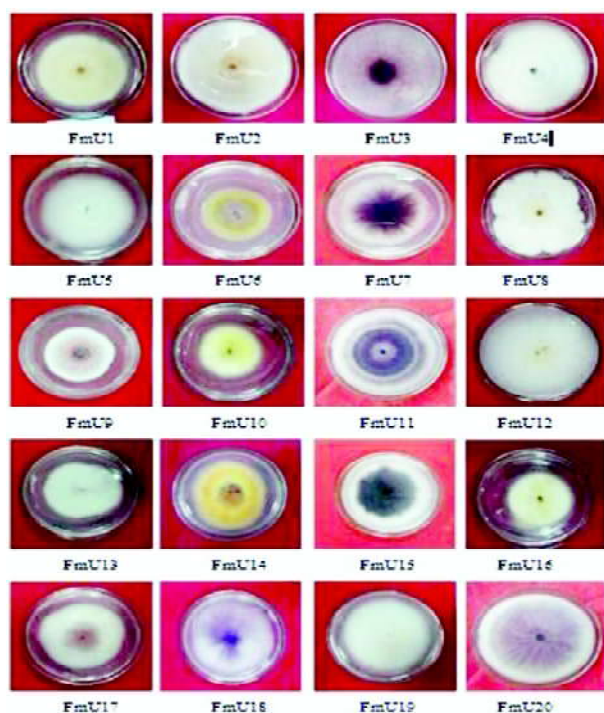


Fig 2: Difference in colony characters and pigmentation in isolates

plate, two from right angle to each other and one from very close to the inoculation point and another at the midpoint of radius using a sterilized needle and transferred onto a clean glass slide. The culture was stained with cotton blue and observed for macro and micro conidial characteristics under microscope.

RESULTS AND DISCUSSION

Survey of Occurrence of Pokkah boeng Disease at Different Locations of District Udham Singh Nagar.

The survey conducted in the month of July as mentioned in the material and methods revealed that the Pokkah boeng disease incidence was found more prominent during rainy season.

Morphological variability

The pure culture of 20 isolates was allowed to grow in Petri plates containing PDA and growth

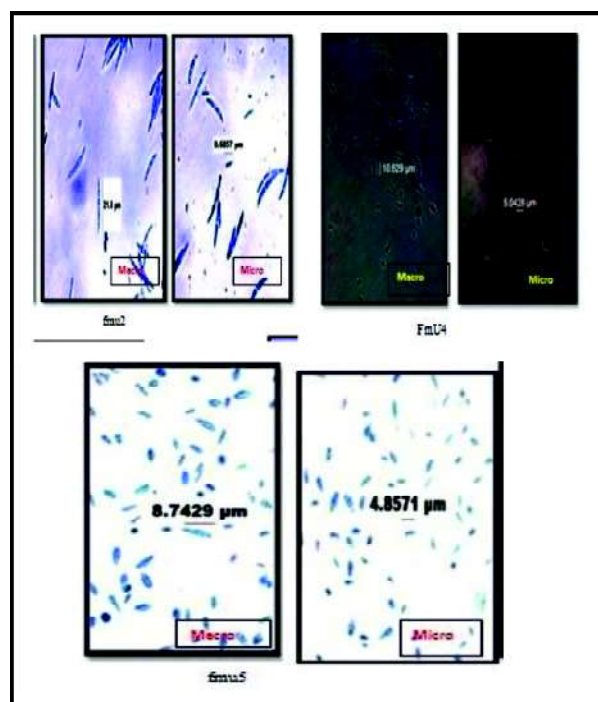


Fig 3: Size of macro and microconidia of isolates Fmu 2 Fmu 4 and Fmu 5, at 40x

of each isolates was measured at 48hrs interval. On the basis of growth rate all the isolates were categorized into 3 categories, fast, medium and slow growing. The maximum colony diameter (90mm) was recorded in isolates FmU2, FmU10, FmU12, FmU17, FmU18 after 10 days of inoculation and categorized as fast growing, while medium colony diameter was observed in FmU3 (85mm), and FmU20 (87mm) FmU1 (71mm), FmU8 (80mm), FmU9 (76mm), FmU11 (81mm), FmU13 (76mm), FmU14 (78mm) and aFmU15 (82mm). Slow growth rate was observed in isolates FmU4 (76mm), FmU5 (68mm), FmU7 (71mm), FmU6 (75mm) and FmU19 (73mm). A wide range of colour variation was observed in isolates on the basis of colony texture and pigmentation. Isolates that differed in their cultural characteristics produces different pigmentation and colony texture. Dark colour pigmentation was observed in isolates FmU3 (salmon pink), FmU 7 (dark meroon), FmU11 (violet), FmU15 (dark brown) whereas light pigmentation was observed in FmU1 (light

yellow), FmU2(light orange), FmU9(peach colour) and no pigmentation was observed in isolates FmU4, FmU5, FmU8, FmU13, FmU19. Colony texture varied from fluffy profuse submerged to cottony and pigmentation pattern was very much similar as described by Sharma and Kumar (2015), he also studied the variability in *Fusarium moniliforme* causal agent of Pokkah boeng and illustrated that isolates showed wide range of pigmentation from whitish cream to rosy pink.

Conidial Characters

To see the variation among conidial characters all the isolates were studied under microscope and found that the fungus produces micro and macro conidia and both varied significantly with respect to size and shape. The conidiophore was hyaline, short and conidia were formed on the apex of the branches. Micro conidia were slightly sickle, oval to clavate shaped and 0-1 septate. Macro conidia were sickle shaped thin walled and abundantly produced. The length of micro conidia ranged from 4.85-8.68 μ m in FmU5 and FmU2. The different isolates showed great degree of variation within different parameters like size of macro conidia, and micro conidia. The largest macro conidia was observed in FmU2 (21.8 μ m) followed by FmU1 (15.54 μ m) and FmU6 (15.24). Macro conidia in different isolates ranged from 21.8- 8.74 μ m and these were hyaline 1-2 celled, oval to club shaped and almost straight to narrow at the ends.

CONCLUSION

Pokkah boeng disease of sugarcane is emerging as the most severe disease in many sugarcane growing areas of the world. A survey was conducted during monsoon season in the month of July-August in major sugarcane growing areas of district Udham Singh Nagar namely Kichha, Sitarganj, Khatima, Nanakmatta, Bazpur, Pantnagar, Jawaharnagar and Kashipur revealed the prevalence of the disease in these areas. The isolates collected from different regions of Udham Singh Nagar were studied for morphological

variability and significant difference was observed in the colony characters in terms of mycelia growth, pigmentation of culture and conidial shape and size which may have difference in the degree of pathogenecity and aggressivity. Further studies on these aspects can help in the identification of more aggressive isolates and could drive our focus for management in regions where they are present.

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