INVESTIGATIONS ON 'TUNDU' OR YELLOW EAR ROT DISEASE OF WHEAT CAUSED BY *CORYNEBACTERIUM MICHIGANENSE* PV. *TRITICI* (HUTCHINSON) DYE AND KEMP AND *ANGUINA TRITICI* (STEINBUCH) FILIPJEV.

by

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Thesis submitted to the Haryana Agricultural University in partial fulfilment of the requirements for the degree of:

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D E D I C A T E D

T O

M Y P A R E N T S

CERTIFICATE I

This is to certify that this thesis entitled "Investigations on Tundu or yellow ear rot disease of wheat caused by *Corynebacterium michiganense* pv. *tritici* (Hutchinson) Dye and Kemp and *Anguina tritici* (Steinbuch) Filipjev" submitted for the degree of Master of Science, in the subject of Plant Pathology, of the Haryana Agricultural University, is a bonafide research work carried out by Shri Ram Mehar Singh Kairon under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE II

This is to certify that the thesis entitled "Investigations on 'Tundu' or yellow ear rot disease of wheat caused by *Corynebacterium michiganense* pv. *tritici* (Hutchinson) Dye and Kemp and *Anguina tritici* (Steinbuch) Filipjev" submitted by Sh. Ram Mehar Singh Kairon to the Haryana Agricultural University in partial fulfilment of the requirements for the degree of Master of Science, in the subject of Plant Pathology has been approved by the Student's Advisory Committee after an oral examination of the same, in collaboration with an External Examiner.

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CONTENTS

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CHAPTER I

INTRODUCTION

Amongst the cereals which must necessarily take the leading position in crops of primary significance to mankind, wheat is the most important because it provides the people of every country with quality nutrient. About half of worlds population depends for sustenance on its production. In India, wheat is grown over 23 million hectares which yield nearly 44 million tonnes of grain (Anon; 1983).

Many kinds of pathogens attack wheat causing greater reduction in yield. The bacterium (*Corynebacterium michiganense* pv *tritici* (Hutchinson, 1917) Dye and Kemp, 1977) in association with the ear-cockle nematode (*Anguina tritici* (Steinbuch, 1799) Filipjev 1936) causes a yellow ear rot disease, which is commonly referred to, in this country, as 'tundu' disease. 'Tundu' is one of the important diseases in India, which has been reported to cause an annual loss of Rs. 75 million even at a low infestation level of less than one per cent (Seshadri, Das Gupta 1980). Singh *et al.* (1953) recorded an annual loss of 30 per cent in U.P. state due to this disease only. Chakravarti and Singh (1961) recorded prevalence of 'tundu' as high as 40 per cent on a local variety in Bihar. Paruthi (1982) reported that the incidence of 'tundu' on ear heads, in Haryana, ranged from 0.31 to 6.96 per cent.

The disease has been disseminated through infested seeds to all the wheat producing regions of the world. In India this disease was first recorded from Punjab (India)

by Hutchinson in 1917; from Egypt by Fahmy and Mikhail, 1925; from western Australia by Carne, 1926.

Although the disease has been studied and reported by various workers from different parts of the world, yet the information about the precise relationship between the nematode and the bacterium is not complete. Chaudhuri (1935) reported that 'tundu' disease of wheat is caused by the bacterium alone. Sabet (1952) suggested that bacterium was present in the soil and carried by the nematode as contaminant. Cheo (1946) suggested that bacterium was carried with in the wheat galls. Gupta (1966) reported that 'tundu' disease is caused by the association of nematode and bacterium and nematode acts as a vector. Detailed studies on the symptoms, histopathology and salient features of the morphology of the various developmental stages of Anguina tritici have been carried out by Gupta and Swarup (1968a,b), Swarup and Gupta (1971) with very little emphasis on the bacterial aspects. For the control of yellow ear rot and ear cockle diseases of wheat, water floatation technique is in practice but continuous occurrence of this disease proved that this method is not so much practically adopted. Vasudeva and Hingorani (1952) compared salt sedimentation, water floatation and heat treatment and found that disease was kept under control by sedimentation and water floatation but solar heat treatment was not effective in destroying the bacteria. In view of economic importance of

the disease, detailed investigations were taken up with the following objectives.

1. To study the association of nematode and bacterium in causing the disease with particular emphassis on the bacterial aspect of the disease complex.

2. Location of bacterium in galls, larvae, plants at different stages of plant growth.

3. To device suitable chemical control measures to minimise the losses caused by the disease.

CHAPTER II

REVIEW OF LITERATURE

The yellow ear rot or 'tundu' disease of wheat was first reported from Punjab (India) by Hutchinson (1917) and subsequently the disease has been reported from various other countries like China, Australia, United Arab Republic, Ethiopia, Egypt, U.S.A., U.S.S.R., Canada, Brazil, New Zealand, Pakistan, Israil and from serveral Europeon countries (Fahmy and Mikhail, 1925; Sabet, 1952, 1954a, 1954b; Carne, 1926, Cheo, 1946; Hingorani and Bekele, 1969).

The disease is characterised by the presence of bright yellow slime or gum on abortive ears and leaves in contact with such ears in the boot leaf. The affected ears fail to produce any grains. The initial symptoms are same for both ear cockle and 'tundu' (Gupta and Swarup, 1968). Seedlings become twisted and crinkled and may die in severe infections. In the later case the emerging spike is either narrow and short with grains partially or completely replaced by bacterial mass, or it fails to emerge out of boot leaf. The stalk is always distorted when ear shows bacterial rot symptoms (Hutchinson, 1917; Milne, 1919; Carne, 1926; Chaudhuri, 1935; Vasudeva and Hingorani, 1952; Sabet, 1954; Gupta, 1966 and Gupta and Swarup, 1968).

Primary sources of inoculum are nematode galls carried in soil as contaminant of wheat seed (Cheo, 1946; Vasudeva and Hingorani, 1952) or soil (Sabet, 1954). The galls carry the bacterium either on the out side surface (Swarup and Singh,

1962) or inside the gall (Gupta, 1966; Gupta and Swarup, 1972). Chaudhuri (1935) reported that bacterium alone could cause the typical disease symptoms, but detailed studies carried out subsequently by various workers showed beyond doubt that the infection could take place only when the nematode galls were mixed with the bacterial inoculum (Vasudeva & Hingorani 1952 a, b; Sabet, 1954). Various workers (Gupta and Swarup, 1968; Swarup and Singh, 1962; Swarup and Gupta, 1971; Midha, 1971) have reported about the various aspects of association of the bacterium Corynebacterium michiganense pv tritici and the nematode Anguina tritici in causing 'tundu' disease.

Fahmy and Mikhail (1925), Carne (1926), Cheo (1946) and Gupta (1966) reported that the bacterium was carried into the plants by nematode. Swarup and Singh (1962) reported that both nematode and bacterium are essential for the expression of 'tundu' symptoms but nematode larvae themselves, however, do not appear to carry the bacterium internally or externally. Pitcher (1963) observed that nematode acts as a vector of the bacterium for carrying it either from part to part of a single plant or from soil to plant. Midha (1971) detected bacterium in the galls collected from even those areas where 'tundu' disease has not been recorded suggesting thereby that the relationship between the two organisms is of complex nature. Galls were reported by him to serve as nutrient source to the bacterium. Bamdadian (1973) observed that the nematode Anguina tritici

acts as a vector of yellow slime disease of wheat caused by A. tritici and C.m. pv tritici and has been found in all samples of disease in Iran.

Fahmy and Mikhail (1925) observed no grain formation in 100 ear heads infected with Corynebacterium tritici. Vasudeva and Hingorani (1952) reported 1-3 per cent damage in Delhi state in general and upto 50 per cent in individual cases. Singh et al. (1953) recorded an annual loss upto 30 per cent in yield due to this disease in U.P. state. Sabet (1954) reported that number of plants infected by eelworm and bacterium were 1 out of 52 and 19 out of 57 respectively. Chakravarti and Singh (1961) recorded prevalence of 'tundu' as high as 40 per cent on a local variety of Bihar. Hingorani and Bekele (1969) reported C. tritici for the first time on wheat from Ethiopia and incidence was as high as 50 per cent in some fields. According to Paruthi and Bhatti (1982) the incidence of 'tundu' on ear heads in Haryana ranged from 0.31 per cent to 6.96 per cent.

Leukel (1924) reported that 'tundu' disease attacks wheat, Rye, emmer (Triticum dicoccum) and spelt (Triticum spelta L.) with almost equal virulence. Bhatti et al. (1978) first time recorded 'tundu' and ear cockle together on barley in Hisar (Haryana). Dahiya & Bhatti (1980) from Hisar (Haryana), reported that Lolium temulentum, Alopecurus monspeliensis and Phalaris minor as hosts of the 'tundu' disease.

Midha and Swarup (1974) reported that the analysis of gall extract disclosed 13 amino acids and one gram of gall extract contained sugars equivalent to 42 mg. of glucose.

Swarup and Gupta (1971) reported that out of 5 media tested for the growth of bacteria, yeast glucose chalk agar medium (YGCA) and special medium supported the best growth.

Sieving is the simplest way for the farmers to achieve gall eradication and is commonly practised in various countries. This method was tested by Coleman and Regan (1918), Jones et al. (1938) and Chu (1945) who found that .05 per cent of the galls remained in the mixture even after sieving. Coleman and Regan (1918) suggested that seed can directly be put in water. Chu (1945) made observation and found that fresh water method will not be effective unless the operator can remove the galls right after the contaminated wheat seed is poured into water. Byars (1919) applied the salt sedimentation method in North America. Different workers have advocated the use of different concentrations of the salt. Hodson (1933) used 30 per cent, Leukel (1929), Singh et al. (1953) and Jain et al. (1956) used 20 per cent concentration. Vasudeva and Hingorani (1952) compared salt sedimentation, water floatation and solar Heat treatment and found that disease

was kept under control by sedimentation and water floatation but the solar heat treatment was not effective in destroying the bacteria. Paruthi and Bhatti (1982) reported that complete floatation of galls was obtained at or above 10 per cent salt solution for 5 minutes.

Srivastava and Katiyar (1956, 1957) reported that among chemicals tested so far, Diazinon (0.15%) has been found effective. Gupta (1966), Midha (1969) found that Zinfos has been found effective in reducing infection. Diazinon has been reported to be phytotoxic. Chakravarti and Rangarajan (1966) reported that streptocycline inhibited the growth of the bacterium under laboratory conditions.

CHAPTER III

MATERIALS AND METHODS

Wheat var. C-306, obtained from Director farm H.A.U. Hisar was used. Wheat sowing was done in screen house on November 11, 1983 and in field on November 26, 1983. The ear cockle galls collected from the infested field from Bawal (Haryana) during 1982-83 were used as inoculum. In pot experiment, field soil was used which was mixed with farm yard manure in the ratio of 3:1.

Isolation of the bacterium:

a) 'Tundu' affected earheads and galls:-

Yeast-glucose chalk agar (YGCA) medium was used for isolation of Corynebacterium michiganense pv tritici. The galls were sterilised with 0.1 per cent mercuric chloride solution for one minute followed by washing in three changes of sterilised distilled water. The galls were then gently curshed in 2-3 ml of sterile distilled water. Isolations were made from this by streak method. Test tube slants were incubated at 27°C for 48 hrs. Isolations from 'tumdu' ear heads were also made in a similar manner. Culture of the bacterium were maintained on YGCA slants at 8°C ± 2°C in refrigerator.

b) Different plant parts:-

For isolation of bacterium, different plant parts viz., growing point, leaves and awns were taken and small pieces were cut with a sterilised scissors. Cut pieces were shaken in

sterilised water and streaked on YGCA slants. In another case the cut pieces were surface sterilised with 0.1 per cent mercuric chloride solution for 30 seconds followed by washing in three changes of sterilised distilled water. These pieces were teased with sterile needle in 2-3 ml. of sterile distilled water. Isolations were made from this by streak method and tubes were incubated at 27°C for 48 hrs.

c) Nematode larvae:-

Isolations for the bacterial presence both on the outer surface and inside of the larvae. Surface sterilised galls were soaked in sterilised water for 24 hours and then pressed with a pair of sterilised foreceps. Isolations from larvae were made to detect the presence of bacterium as contaminant on the outer surface. For surface sterilisation the larvae were treated with 0.1 per cent mercuric chloride for 30 minutes. The larvae suspension was poured on a 400 mesh sieve and washed with three changes of sterilised distilled water. Larvae from the sieve were collected in separate Petri plates in small quantity of sterilised water and the larvae were crushed. Suspension was streaked on the YGCA slants and incubated at 27°C for 48 hrs.

The bacterial culture was purified by repeated single colony transfer. The isolated bacterial culture was identified on the basis of their morphological and biochemical tests as given in Mannual of Microbiologist Methods (Society of American Bacteriologist, 1957 and Bergey's, Manual of Determinative Bacteriology, 1974).

Composition of different media used are given below:-

a) Yeast glucose chalk agar medium (YGCA):-

Yeast extract	= 10.0g
Calcium carbonate	= 20.0g
Glucose	= 10.0g
Agar	= 20.0g
Distilled water to make 1000 ml.	

b) Nutrient agar:-

Peptone	= 5.0g
Beaf extract	= 3.0g
Agar	= 20.0g
Distilled water to make 1000 ml.	

c) Nutrient agar + gall extract:-

Peptone	= 5.0g
Beaf extract	= 3.0g
Agar	= 20.0g
Gall extract	= 5.0g
Distilled water to make 1000 ml.	

d) Gall extract medium:-

Agar	= 20.0g
Gall extract	= 10.0g
Distilled water to make 1000 ml.	

All these media were adjusted to PH 7.8 by addition of N/10 Na OH or N/10 HCl and sterilised at 15 lbs pressure for

20 minutes. Bacterium was inoculated in the different sterilised media and incubated at 27°C. 48 hours growth containing the bacteria were serially diluted and aliquotes of each dilution were placed on YGCA culture medium in Petri plates. Colonies developing on each plate were counted. Number of bacterial cells in an 48 hrs. growth in one slant were calculated by multiplying the average number of colonies per countable plate by the reciprocal of the dilution and the results reported as bacterial cells per 48 hrs. growth in a slant.

Inoculation of Plants (Bacterium, nematode in different combinations).

a) At sowing time:-

Two galls per seed were used as inoculum at the time of sowing. 'Tundu' affected ear heads were crushed and powder was mixed with the galls to provide sufficient natural bacterium inoculum where ever required. For the inoculation with nematodes only, sterilised larvae suspension was poured around the seed at the time of sowing. For inoculations with bacterium alone the bacterial cell suspension and infected ear heads powder were used. Wheat seeds were sown in sterilised soil to serve as control.

b) At different stage of plant growth:-

Bacterial cell suspension 8.1×10^6 cells/ml was prepared by suspending 48 hrs. bacterial growth on YGCA slant

in 100 ml sterilised water. Before inoculation very minute injuries were given to the plants with the help of pin head and then bacterial cell suspension was sprayed with an automizer. Some plants were injected with bacterial cell suspension. After inoculation plants were covered with polythene sheet for 24 hours to provide sufficient humidity. The development of symptoms were studied after 10 days of inoculation in each case till maturity.

Analysis for amino acids.

The nematode galls weighing 10 g were soaked in 10 ml of 80 per cent of ethanol for 24 hrs. in seprate container and allowed to boil for 5-10 minutes. The extract was then cooled and the galls and wheat seeds were crushed thoroughly, passed through two layers of cheese cloth and re-extracted for 3 minutes in 80 per cent boiling ethanol. The extract was filtered through filter paper.

2.5 g galls were extracted in 25 ml of 80 per cent ethanol. The ethanol was then evaporated by boiling the extract on the top of steam bath. The residues were dissolved in sterilised distilled water to make the final volume to 25 ml.

Descending paper chromatographic technique of Block et al. (1958) was employed. 5 µl of each sample was spotted on Whatman chromatographic paper No. 1 ($22\frac{1}{2}$ x $18\frac{1}{2}$") with the help of micro pipettes. The solvent used was nbutanol-acetic

acid-water (4:1:5). The chromatograms were then taken out, dried and 0.2% ninhydrin dissolved in acetone was used for the detection of free amino acids. Rf values were calculated and compared with those of known amino acids which were also run simultaneously. The Rf. values were calculated by the formula:-

$$\text{Rf. value} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Screening of varieties for disease resistance:-

Twenty one wheat varieties/lines were screened for their relative resistance to 'tundu' disease in the field under artificial inoculation conditions.

Seeds of each of the variety/line were sown in the field in second week of December during the year 1983. Inoculation were done at the time of sowing by placing two galls with each seed.

Observations were recorded periodically till maturity of the crop. 'Tundu' ear heads and number of galls formed in each treatment were also counted.

Efficacy of antibiotics and their mixture with insecticides in inhibition of bacterial growth in vitro:-

YGCA medium was poured in each of the sterilised petri plate. .5 ml bacterial cell suspension was poured in each Petri plate with the help of a sterilised pipette and

spread with the help of glass rod to obtain a uniform spread of the bacterium. The Petri plates were refrigerated for 1 hour. In the mean time antibiotics and insecticidal solutions (conc. 1000 ppm) were prepared in sterile distilled water. The sterilised filter paper discs were immersed in different solutions. Filter paper disc was touched twice to dry inside the glass tube containing the solution to remove any excess solution and the disc was placed on the agar surface of one of the plates. The same procedure was followed for other antibiotic solutions, laying one disc of each sample in the centre of separate Petri dish. The Petri plates were incubated at 27°C for 48 hrs. The diameter of the zone of inhibition was measured.

Efficacy of antibiotics, insecticides, nematicides and their combinations in 'tundu' disease control.

The following were used.

Common Name	Chemical Name	Source
Streptocycline	Streptomycin+Chlorotetracycline	Hindustan antibiotic Ltd.
Paushamycin	Streptomycin 15 per cent + oxytetracycline 1.5 per cent	Paushak Ltd; Alembic Road, Baroda.
Rogor	0,0 - Dimethyl 5-(N-methylcarbamoylmethyl) Phosphorodithioate	Rallis India.
Anthio	0-0-Dimethyl 5-(N-methyl-N-formy -lcarbamoylmethyl).	Sandoz.

Nuvacron	Dimethyl phosphate of 3-hydroxy - N - methyl-cis-crotonamide.	Hindustan Ciba Giegy.
Carbofuran	(dihydro 2,2-dimethyl benzafuran)-7-yl methyl carbamate.	Rallis India.
Nemacur	Ethyl 3-methyl-4-(methylthio) phenyl (1-methylethy) phosphoramidate.	Bayer (India) Limited.
Dimecron	2-chloro-2 diethyl carbamoyl -1- methylvinyl dimethyl phosphate phosphorodithioate.	Hindustan Ciba Giegy.
Phorate	0,0-diethyl S-(ethyl thiomethyl).	Cyanamid (India) Ltd.
Metasystox	(Mixture of Thiono and Thiolo isomers) 0,0-Dimethyl 0-2 ((ethylthio) ethyl) Phosphorothioate.	Bayer (India) Limited.

Seed used for sowing was mixed with nematode galls (2 galls/seed) before sowing in the field. Wheat seeds were soaked in Nemacur 40 E.C. 0.1%, Streptocycline 1000 ppm, Paushamycin 1000 ppm and Paushamycin + Nemacur solution for 20 minutes.

For soil application Nemacur 5 G., Phorate 10%, Carbofuran 3% were broadcasted in individual plots before sowing @ 1.5 kg a.i./ha.

Foliar application was done twice with antibiotics viz., Streptocycline 1000ppm, Paushamycin 1000 ppm, Nemacur E.C. 0.1% conc., Metasystox 25 E.C. 0.1% conc., Rogor 25 E.C. 0.1% conc., Anthio 25 E.C. 0.1% conc., Dimecron 100 E.C. 0.1% conc., Nuvacron 0.1% and their combinations with antibiotics on wheat plants. First spray was given 20 days after sowing and second one month after the first spray. Plants were harvested at maturity and each ear head was examined for the presence of infection with 'tundu' or earcockle.

CHAPTER IV

EXPERIMENTAL RESULTS

Symptomatology:-

In the initial stages of infection, leaves of wheat seedlings showed a bright yellow slime on its surface. In many cases, a pale yellow streak running parallel to the veins was also observed to be produced by the bacterium on leaves (Plate 1).

Twisting and crinkling of leaves has been observed to be a very common symptom as a result of infection by the nematodes alone and also when bacterium was associated with it. In addition to yellowing of leaves, enlargement of the basal stem portion in young seedlings (20 days old) was also observed. Wheat seedlings mortality have been observed under severe infections with the bacterium. Generely severely infected plants failed to develop any ear heads.

Yellow slime was also observed on the emerging ears and leaves in contact with them at boot leaf stage. The stalk was quite often distorted when ears developed typical 'tundu' symptoms. Emerging ears were always narrower and shorter with the grains partially or completely replaced by the bacterial mass and were typical yellow in appearance (Plate 2). Awns, if developed were also covered with yellow slimy bacterial mass.

In the ears, all or some of the grains were found to be replaced by cockles (galls) which on drying became round, hard, dark brown to black in colour.

PLATE 1

A pale yellow streak produced by the bacterium on wheat leaves.

PLATE 2

Wheat plants showing 'tundu' disease symptoms.

Isolation and identification of the bacterium:-

The bacterium was isolated from slimy ear heads on yeast glucose chalk agar medium. It was found to be gram positive and having short rods (0.8 by 2.4 to 3.2 microns) and motile by means of single polar flagellum. It grew well on yeast glucose chalk agar medium and produced bright yellow, glistening, opaque, moist, smooth colonies (Plate 3). However, on nutrient agar medium growth of the bacterium was slow with light yellow colour colonies. Negative for hydrogen sulphide production, produced nitrites from nitrates, produced acid but no gas on glucose and lactose.

In all the isolations from 'tundu' ear heads, another bacterium was consistently isolated alongwith the pathogen. This produced orange coloured colonies on the medium and was found to be gram negative. From further studies it was found not to be antagonistic to the pathogen.

Detection of the bacterium in different plant parts:-

Wheat seed mixed with Nematode galls and crushed 'tundu' ear heads were mixed with wheat seeds and sown in pots containing sterilised soil. Isolations made from different plant parts at different stages of growth on YGCA medium showed the presence of the bacterium (Corynebacterium michiganense pv tritici) in the growing point, leaves, stem and awns (Table 1).

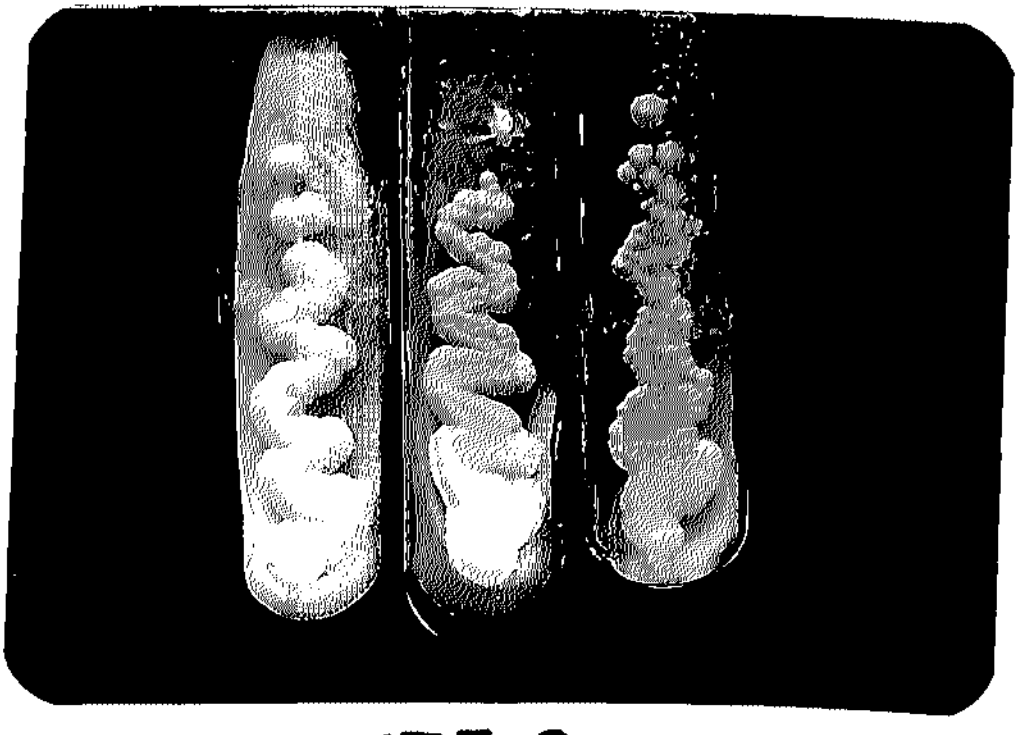

PLATE 3

Corynebacterium michiganense pv tritici growth on yeast glucose chalk agar medium.

Table 1: Isolation of C.m. pv tritici from plant parts and galls.

Treatment	Presence/absence of bacterium
Growing point	+
Leaf	+
Stem	+
Awns.	+
Unsterilised galls(outer surface)	-
Unsterilised larvae	+
Sterilised larvae	-

+ 'Present' - 'Absent'

It is clear from the results presented in Table 1 that bacterium was not present on the surface of the galls. It indicate that bacterium remains inside the galls. Isolations made from the unsterilised larvae reveal the presence of the bacterium on the surface of larvae. The bacterium could not be isolated from the larvae sterilised in mercuric chloride solution for 30 minutes. It indicate that nematode carried the bacterium on the surface and not internally.

Incidence of bacterium in nematode galls of different location:-

Galls were collected from three different locations and isolations were made to find out the extent of bacterial infestation. The results are presented in Table 2.

Table 2: Incidence of Corynebacterium michiganense pv tritici in nematode galls from different locations.

Locations	Percentage of galls with C.m. pv tritici
Hisar	70.00
Bawal	73.33
Rewari	83.33

It was observed that 70.00 to 83.33 per cent galls carried the bacterium. The maximum number of galls which carried the bacterium were collected from Rewari (83.33%) followed by Bawal (73.33%) and Hisar (70.00%).

Effect of nutrient media on growth:-

Four different media viz., yeast glucose chalk agar, nutrient agar, nutrient agar + gall extract and gall extract were used to study whether the galls provided sufficient nutrition for the growth of bacterium or not. The results are presented in Table 3.

Table 3: Effect of different culture media on the growth of C.m. pv tritici.

Media	Bacterial cells/ml (48 hrs. growth in 100 ml water)
Agar + Gall extract	2.1×10^4
Nutrient agar	2.9×10^4
Nutrient agar + gall extract	1.86×10^5
Yeast-glucose chalk agar	8.1×10^6

It is clear from Table 3 that the maximum growth was recorded on YGCA (8.1×10^6 cells/ml) followed by nutrient agar + gall extract (1.86×10^5 cells/ml), nutrient agar (2.9×10^4 cells/ml) and gall extract agar medium (2.1×10^4 cells/ml). It indicate that gall extract provides the nutrients for the growth of the bacterium. However yeast glucose chalk agar medium was found to be the best medium for this pathogen.

Inoculation of plants (Bacterium, nematode in different combination:-

To study the role of bacterium, nematode and their combination in the production of disease, plants were inoculated with different types of inocula. The results are presented in Table 4.

It is clear from the results presented in Table 4 that typical symptoms of 'tundu' appeared only in those plants in which unsterilised galls and sterilised galls were used as inoculum. Typical disease symptoms also appear when unsterilised larvae were added to the soil around the roots of plant. However when nematode or bacterium inoculum was added seprately, there was no expression of 'tundu' disease. Surface sterilised larvae produced ear cockle symptoms only, where as, bacterial symptoms failed to appear when plants were inoculated with bacterium at any growth stage of the plants.

Table 4: Different inoculum sources in relation to 'tundu' and ear cockle incidence.

Treatment	Seedlings showing crinkling (%)	Seedlings showing yellowing (%)	Plants with 'tundu' ears (%)	Yield/ plant (gm.)	No. of galls/ 1000 grains.	Absence/ presence of bacterial in galls
1. Sterilised galls in sterilised soil.	11.53	11.53	11.53	11.88	2.08	+
2. Unsterilised galls in sterilised soil.	8.69	13.04	17.39	8.86	3.10	+
3. Unsterilised galls+bacterium in sterilised soil.	7.14	14.28	8.58	9.46	2.66	+
4. Sterilised galls+bacterium in unsterilised soil.	16.66	20.83	8.33	6.95	4.40	+
5. Sterilised larvae in soil	7.69	0	0	13.88	0.63	-
6. Unsterilised larvae in soil.	15.38	11.53	7.69	9.53	3.70	+
7. Sterilised larvae+bacteria in unsterilised soil.	3.57	7.14	7.14	8.78	1.68	+
8. Bacteria in unsterilised soil	0	0	0	15.88	0	-
9. Sterilised gall+Bacteria sprayed on young seedlings	6.89	13.49	13.79	7.93	2.40	+
10. Sterilised galls+Bacteria inoculated at ears emergence	11.53	3.94	11.16	14.42	3.14	+
11. Bacteria inoculated on ears	0	0	0	14.00	0	-
12. Control, wheat seed in sterilised soil	0	0	0	16.72	0	-
13. Control, wheat seed in unsterilised soil.	0	0	0	16.68	0	-

Of the thirteen different inoculation treatments disease was expressed only in eight treatments viz., sterilised galls in sterilised soil, unsterilised galls in sterilised soil, unsterilised larvae in soil, sterilised larvae plus bacterium in unsterilised soil, sterilised galls plus bacterium sprayed on young seedling and sterilised galls plus bacterium at ear emergence. In the remaining treatments viz., sterilised larvae in unsterilised soil, bacterium on ear emergence and control in sterilised and unsterilised soil 'tundu' disease symptoms did not appear. These results clearly indicate that typical 'tundu' disease symptoms appear only when both nematode and the bacterium are associated. This is also evident that source of bacterium inoculum is the galls which carry the bacterium inside. Since no disease symptom were noticed when sterilised larvae were used as inoculum as compared with unsterilised larvae which resulted in typical 'tundu' symptoms indicate that larvae carried the bacterium to the plants on their surface.

Analysis for amino acids in galls:-

Galls extract was prepared in 80 per cent ethanol. The galls were analysed by descending paper chromatographic technique. The results of the qualitative analysis of amino acids in galls are given in Table 5.

Table 5: Amino acids detected in galls.

Amino acids	Gall
1. DL - Valine	+
2. DL - Serine	+
3. DL - Alanine	+
4. DL - Tryptophan	+
5. DL - Threonine	+
6. L - Tyrosine	+
7. L - Arginine monohydrochloride	+
8. DL - Methionine	+

It is clear from the Table 5 that eight amino acids viz., DL - Valine, DL - Serine, DL - Alanine, DL - Tryptophan, DL - Threonine, L - Tyrosine, L- Arginine monohydrochloride and DL - Methionine were detected in the galls. It indicate that galls provide nutrition for bacterium.

Screening of varieties/lines for disease resistance:-

Twenty one wheat varieties/lines were screened against 'tundu' or yellow ear rot disease in the field under artificial inoculation conditions. Galls and crushed 'tundu' ear heads were mixed with seed at the time of sowing. The observations on the incidence of disease were recorded periodicaly. The results are presented in Table 6.

Table 5: Amino acids detected in galls.

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Screening of varieties/lines for disease resistance:-

Twenty one wheat varieties/limes were screened against 'tundu' or yellow ear rot disease in the field under artificial inoculation conditions. Galls and crushed 'tundu' ear heads were mixed with seed at the time of sowing. The observations on the incidence of disease were recorded periodicaly. The results are presented in Table 6.

Table 6: Screeing of different varieties/lines of wheat for resistance against 'tundu' disease of wheat.

Varieties	Tillers with 'tundu' ear heads (%)
DWR 39	17.3
DWL 5023	15.3
WH 147	49.0
WH 147 (M)	38.1
WH 147-2	17.8
WH 147-3	30.3
WH 157	5.5
WH 283	35.1
WH 291	15.2
WH 331	20.0
HD 1011	0.0
HD 2009	23.3
HD 2281	16.3
HD 2285	19.5
HD 2329	10.9
R 1555	0.0
R 1972	31.6
Frontona (1) 3P	0.0
C-306	18.1
NT 5439	29.8
Kalyan Sona	17.0

It is clear from the results presented in Table 6 that out of 21 wheat varieties/lines screened, three varieties viz., HD 1011, Frontona (1)3P and R 1555 were found free of infection. Varieties/lines viz., WH 157 and HD 2329 were found resistant against the disease. However, varieties/lines viz., WH 147(M), WH 147-3, WH 283, NT 5439 and R 1972 behaved as susceptible and all other varieties/lines behaved as moderately resistant to moderately susceptible.

In-vitro antibacterial efficacy of antibiotics and their combinations with insecticides:-

The effect of two antibiotics (Streptocycline and Paushamycin) and Their mixtures with insecticides (Dimecron, Nuvacron, Anthio and Metasystox) on Corynebacterium michiganense pv tritici growth inhibition was studied by paper disc inhibition zone method. The results are presented in Table 7.

Table 7: Effect of antibiotics and insecticides on the growth of C.m. pv tritici in vitro.

Treatment	Growth inhibition zone (mm)*
Streptocycline	38.5
Streptocycline + Dimecron	46
Streptocycline + Nuvacron	51.5
Streptocycline * Rogor	50
Streptocycline + Metasystox	45.5
Paushamycin	47
Paushamycin + Dimecron	49.5
Paushamycin + Nuvacron	49.5
Paushamycin + Rogor	44
Paushamycin + Metasystox	43
Control	0

*(Average of three replications)

It is clear from the Table 7 that when two antibiotics tested alone, Paushamycin was more effective in checking the growth of bacterium as compared to Streptocycline. In case of Paushamycin, the inhibition zone was 47 mm where as in case of Streptocycline it was 38.5 mm. The effect of these two antibiotics with Dimecron, Nuvacron, Rogor and Metasystox seprately, was also studied.

Maximum inhibition zone was recorded in case of Streptocycline + Nuvacron followed by Streptocycline + Rogor, Paushamycin + Dimecron, Paushamycin + Nuvacron, Streptocycline + Dimecron, Streptocycline + Metasystox, Paushamycin + Rogor and Paushamycin + Metasystox. It was observed that when Streptocycline was mixed with Nuvacron and Rogor, the effectiveness increased as compared to Paushamycin + Nuvacron and Paushamycin + Rogor. Although, Streptocycline was less effective as compared to Paushamycin when tested alone.

Efficacy of antibiotics, insecticides and their combinations in 'tundu' disease control:-

a) Seed treatment:- Wheat seed were soaked in nematicide, antibiotics and mixture of nematicide + antibiotics solution for 20 minutes. The observations were recorded periodically. The results presented in table 8, reveal that all the treatments were superior to control in reducing the seedling crinkling, yellowing, per cent plants with 'tundu' ears and

Table 8: Effect of seed treatment with antibiotics and nematicide on incidence of 'tundu' disease of wheat.

Treatment	Seedlings showing crinkling (%)	Seedlings showing yellowing (%)	Plants with 'tundu' ears (%)	Yield/ plant in gms.	No. of galls/ 1000 grains
Nemacur 40 E.C. 0.1%	5.37	2.15	1.07	13.41	16
Streptocycline 1000 ppm	8.33	7.71	7.14	12.26	13
Paushamycin 1000 ppm	11.42	7.61	4.76	9.71	0
Nemacur + Paushamycin 0.1% + 1000 ppm	18.69	6.14	3.73	11.49	0
Control	46.15	7.69	7.69	11.73	18

number of galls. Seed treatment with Nemacur was found best in reducing the yellowing and 'tundu' ears followed by Nemacur + Paushamycin, Paushamycin alone and Streptocycline. However, Streptocycline was found superior in reducing crinkling of seedlings as compared to Nemacur + Paushamycin. Although Nemacur + Paushamycine was found superior in reducing yellowing and 'tundu' ears in comparison to Streptocycline, but they were found less effective in reducing crinkling as compared to Streptocycline. There was no gall formation in Paushamycin and Nemacur + Paushamycin, where as in case of Nemacur gall formation was high (16 galls/1000seed) followed by Streptocycline (13 galls/1000seeds) and control (18 galls/ 1000seeds). Maximum yield was recorded in Nemacur followed by Streptocycline and control. However, yield was minimum in Paushamycin and Nemacur + Paushamycin, although there was no gall formation in both the cases. Nemacur which was most effective in reducing crinkling, yellowing of seedlings, 'tundu' ears and in increasing yield of grains, supported maximum number of galls in comparison to other treatments. Streptocycline which was found quite effective in reducing crinkling of seedlings supported high percentage of seedling yellowing, plants with 'tundu' ears, number of galls and yield of grain per plant as compared to other treatments.

Soil treatment: Nematicides and insecticides were broadcasted in individual plots at the time of sowing and the observations were recorded periodicaly. The results presented in Table 9,

Table 9: Effect of soil treatment with insecticides and nematicide on incidence of 'tundu' disease of wheat.

Treatment	Seedlings showing crinkling (%)	Seedlings showing yellowing (%)	Plants with 'tundu' ears (%)	Yield/ plant (gms.)	No. of galls/ 1000 grains
Nemacur 5 G. (1.5 kg a.i./ha)	3.70	1.85	1.85	12.06	0
Carbofuran 3% (1.5 kg a.i./ha)	3.70	2.02	0.74	8.40	0
Phorate 10% (1.5 kg a.i./ha)	6.82	3.40	1.46	6.90	0
Control	46.15	7.69	7.69	11.73	18

reveal that crinkling and yellowing was minimum in Nemacur, Carbofaran followed by Phorate and control. However, plants with 'tundu' ears were minimum in case of Carbofuran followed by phorate and Nemacur. Maximum yield was also recorded in Nemacur followed by control, Carbofuran and Phorate. Although there was no gall formation in case of Carbofuran and Phorate, the yield was less even as compared to control. However all these treatments were found quite effective in controlling the crinkling, yellowing and 'tundu' incidence.

c) Foliar application:- Foliar application was done twice with antibiotics, nematicide, insecticides and with their combinations with antibiotics on wheat plants. First spray was given 20 days after sowing and second one month after the first spray. The observations were recorded periodicaly. The results presented in Table 10, reveal that all the treatments were superior to control in reducing seedling crinkling, yellowing and 'tundu' incidence except Paushamycin + Nuvacron in case of seedling yellowing. Nemacur was the best in reducing crinkling followed by Streptocycline, Paushamycin, Paushamycin + Dimecron, Dimecron, Streptocycline + Anthio, Anthio and Paushamycin + Rogor. However in case of seedling yellowing Paushamycin was the best followed by Streptocycline, Streptocycline + Nuvacron, Metasystox, Paushamycin + Nemacur and Anthio. However, Paushamycin + Nuvacron treatment showed maximum number of seedlings showing yellowing. Plants with 'tundu' ears were

Table 10: Effect of foliar application with insecticides, nematicides and antibiotics on incidence of 'tundu' disease of wheat.

Treatment	Seedlings showing crinkling (%)	Seedlings showing yellowing (%)	Plants with 'tundu' ears (%)	Yield/ plant (gms.)	No. of galls/ 1000 grains.
Streptocycline 1000 ppm	6.00	1.41	1.41	5.75	78
Paushamycin 1000 ppm	6.33	0.70	0.70	4.33	14
Nemacur 40 E.C. 0.1%	5.98	4.56	2.81	4.04	13
Rogor 0.1%	10.50	5.07	3.07	4.94	30
Dimecron 0.1%	7.91	2.86	3.59	5.07	56
Paushamycin+Namacur 1000 ppm	11.48	1.90	6.12	6.60	40
Metasystox 0.1%	12.82	1.02	3.58	6.30	0
Anthio 0.1%	9.44	2.22	4.44	7.37	38
Nuvacron 0.1%	10.21	7.28	7.29	7.77	0
Streptocycline+Rogor 1000ppm+0.1%	10.25	8.33	5.76	6.18	0
Streptocycline+Dimecron 1000ppm+0.1%	11.11	3.69	3.70	6.51	0
Streptocycline+Metasystox1000ppm+0.1%	12.69	5.75	2.38	7.93	0
Streptocycline+Anthio 1000ppm+0.1%	9.14	4.56	1.71	5.77	0
Streptocycline+Nuvacron 1000ppm+0.1%	16.36	1.86	3.01	7.19	0
Paushamycin+Rogor 1000ppm+0.1%	9.83	3.26	3.82	7.56	0
Paushamycin+Dimecron 1000ppm+0.1%	7.25	7.25	1.61	7.36	43
Paushamycin+Metasystox 1000ppm+0.1%	13.27	7.95	2.65	7.56	3
Paushamycin+Anthio 1000ppm+0.1%	27.90	6.97	4.65	14.41	13
Paushamycin+Nuvacron 1000ppm+0.1%	45.16	9.00	2.90	18.33	0
Control	46.15	7.69	7.69	11.73	18

minimum in case of Paushamycin followed by Streptocycline, Paushamycin + Dimecron and Streptocycline + Anthio.

There was no gall formation in case of Metasystox, Paushamycin + Nuvacron, Nuvacron, Streptocycline + Rogor, Streptocycline + Dimecron, Streptocycline + Metasystox, Streptocycline + Anthio, Streptocycline + Nuvacron, and Paushamycin + Rogor. However, maximum number of galls were recorded in Streptocycline followed by Dimecron, Paushamycin + Dimecron, Paushamycin + Nemacur, and Anthio. Maximum grain yield was recorded in case of Paushamycin + Nuvacron followed by Paushamycin + Anthio. Although these treatments supported maximum crinkling and yellowing of seedlings. The grain yield/plant was less in all other treatments as compared to control, although there was no gall formation in most of the treatments. Paushamycin and Streptocycline were found quite effective in reducing crinkling, yellowing and plants with 'tundu' ears but grain yield was minimum in both the treatments and maximum galls were recorded in case of Streptocycline.

CHAPTER V

DISCUSSION

The 'tundu' or yellow ear rot caused by Corynebacterium michiganense pv tritici in association with nematode, Anguina tritici is still one of the important disease of wheat. Though the disease has been studied and reported by various workers from different parts of the world yet the information about the precise relationship between the nematode and the bacterium is not complete. Lot of information is however available about the nematode (ear-cockle) aspect of the disease complex. The present study was conducted to study various aspects of the disease like symptomatology, presence of bacterium in different plant parts at various stages of plant growth, extent of bacterial contamination in galls, screening of wheat varieties for resistance and evaluation of nematicides/antibiotics and insecticides and their combination for disease control. The results are discussed in this chapter.

The bacterial streak on leaves was reported by Vasudeva and Hingorani (1952) and Gupta and Swarup (1968). In present study we also observed pale yellow streak running parallel to the veins. Production of temporary local lesion on artificial inoculation was reported by Sabet (1954) but Gupta and Swarup (1968) did not find such lesion. In present study we also did not observe local lesion on artificial inoculation. Yellow slime was also observed on ears and stalk was quite often distorted when ears developed

typical 'tundu' symptoms. Similar observation were recorded by Gupta and Swarup (1968).

Twisting and Crinkling of leaves, as a result of infection by nematode have been discribed by Carne (1926), Chaudhuri (1935), and Cheo (1946). Enlargement of basal swelling was reported by Gupta and Swarup (1968). This was generally found to be ture in the present case as well. Isolations made from different parts of plants raised from seed mixed with nematode galls and bacterium clearly revealed that bacterium was found on growing point, leaves, leaf sheath, stem and awans.

Presence of bacterium on growing point of wheat seedling infected with A. tritici may be due to presence of larvae contaminated with bacterium. Isolations made from plants inoculated with unsterilised galls, outer surface sterilised galls and sterilised larvae showed the presence of bacterium. Only when unsterilised larvae were used as inoculum, the bacterium was isolated from plants. This study clearly shows that bacterium is present on the surface of nematode inside the galls. These results are in agreement with Cheo (1946) and Gupta and Swarup (1972) but in contrary to Singh and Swarup (1962) who reported that the bacterium is present on the surface of the galls.

Presence of bacterium in galls collected from different locations ranged from 70.00 to 83.33 per cent.

High contamination percentage of galls clearly suggest that the galls are the main source of bacterial inoculum. Similar observations were recorded by Pathak and Swarup (1984) but the per cent contamination in our studies was higher (70.00-83.33%) than that observed by them (40-50%). This variation in per cent contamination of galls may be due to galls of different varieties, locations and different environmental conditions.

The result pertaining to effect of different nutrient media on growth of bacterium showed maximum number of bacterial cell/slant in Y.G.C.A. and minimum in agar + gall extract. Gupta and Swarup (1968) also observed maximum growth of bacterium on Y.G.C.A. The results in present study clearly showed that the bacterial growth in nutrient agar was far less than that of Nutrient agar + gall extract. This higher number of bacterial cells in nutrient agar + gall extract clearly shows that gall extract enhances the growth and development of bacterium.

The pathogenecity experiment showed that C.m. pv tritici only produced typical symptoms on wheat plants with the association of the nematode A. tritici. The results are in agreement with Cheo (1946), Vasudeva and Hingorani (1952), Swarup and Singh (1962), Gupta (1966) but in contrary to Chaudhury (1935) who reported that bacterium alone could cause typical disease symptoms.

From wheat seed galls eight amino acids were detected where as Midha and Swarup (1974) reported that the analysis of galls extract disclosed 13 amino acids.

Difficult situation with regard to control of 'tundu' and ear cockle disease in the field is well known therefore, considering the need for a source of resistant varieties, 21 promising and commonly grown varieties were screened under field conditions against this disease. Of the varieties tested three namely HD 1011, R 1515, Frontona (1)3P had no infection and two varieties viz., WH 157 and HD 2329 were found resistant against disease, where as others were found to be moderately resistant to moderately susceptible. These varieties can be used for further breeding programme. Similar observation were reported by Gupta (1966), Midha (1969) and Bhati and Dalal (1975). Similarly according to Joshi et al. (1970) dwarf wheat varieties viz., Lerma Rajo, Sonara-64 and Kalyan Sona were tolerant to Anguina tritici.

In chemical control experiment the efficacy of different pesticides (Insecticides, nematicides and antibiotics) as seed treatment, soil treatment and foliar application was tested. The latter two methods (soil application and foliar application) proved better than the formar one (seed treatment). Because the disease in soil treatment and foliar application with different chemical

was less than that of seed treatment. Carbofuran was found effective in reducing the number of 'tundu' ears. Midha (1969) reported that amongst the nematicides tested, Temik proved to be an effective chemical as a soil application. In present study Carbofuran, Phorate and Nemacur were found effective in controlling the seedling crinkling, yellowing, 'tundu' ears. There was no gall formation in all the cases. However the yield was less as compared to control.

CHAPTER VI

SUMMARY

'Tundu' or yellow ear rot disease of wheat caused by Corynebacterium michiganense pv tritici and Anguina tritici has been investigated. The symptoms in wheat plants effected with 'tundu' disease have been described. In the intial stage leaves of wheat seedlings showed bright yellow slime on its surface. Pale yellow streak running parallel to the veins were also produced by the bacterium. In severe infections heavy seedling mortality was observed. Yellow slime was also observed on emerging ears and the stalk was quite often distorted. Emerging ears were with the grains partially or completely replaced by bacterial mass. The first visible symptom in infected plant by nematode was slight swelling at the base which was followed by twisting and crinkling of the leaves. On the ears, all or some grains were found to be replaced by cockles (galls).

Bacterium was detected on the growing point, leaves, stem and awns. Isolations made from the sterilised and unsterilised larvae revealed the presence of bacterium only on the external surface of the larvae. Incidence of the bacterium in the mematode galls collected from different locations was found maximum in Rewari and the minimum in galls obtained from Hisar.

Yeast glucose chalk agar medium supported the best growth of the bacterium. Galls have been found to provide sufficient nutrition for bacterial growth and development.

Nematode and bacterium are essential for the expression of 'tundu' or yellow ear rot disease of wheat. It was observed that typical symptoms of 'tundu' appeared only when plants were inoculated with unsterilised galls or outer surface sterilised galls or unsterilised larvae. Surface sterilised larvae produced ear-cockle symptoms only. Where as, bacterial symptoms also failed to appear when plants were inoculated with bacterium alone.

Qualitative analysis of gall extract revealed the presence of 8 amino acids. Of the varieties/lines tested for resistance against 'tundu' or yellow ear rot disease three varieties, namely HD 1011, Frontona (1) 3P and R 1515 were found free of infection and WH 157 and HD 2329 were found resistant against the disease.

Effect of antibiotics and their mixture with insecticides on C. michiganense pv tritici was studied and found that inhibition zone size was maximum when Streptocycline was mixed with Nuvacron followed by when Paushamycin was mixed either with Dimecron or Nuvacron.

Nematicides, antibiotics, insecticides and their combinations were used as seed treatment, soil application and foliar application. Seed treatment with Nemacur was found effective in reducing the yellowing, crinkling and 'tundu' ears.

In soil treatment crinkling and yellowing was minimum in Nemacur, Carbofuran followed by Phorate. However 'tundu' ears were minimum in Carbofuran followed by Phorate. There was no gall formation in Nemacur, carbofuran and Phorate.

In foliar application all the treatments were superior to control in reducing seedling crinkling, yellowing and 'tundu' incidence except Paushamycin + Nuvacron. Nemacur was the best in reducing crinkling. However in case of seedling yellowing and 'tundu' ears Paushamycin was the best-

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* Original not seen.