

Stalk Rot of Maize Diseases in the Intermediate Zone of Jammu Region

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ABSTRACTS: Stalk rot of maize caused by *E. chrysanthemi* pv. *chrysanthemi* is one of the most destructive disease in several maize growing regions of India. Most of the maize hybrids and composite released in the country as well as local varieties have been found highly susceptible to this diseases. The intermediate zone covering Poonch and Rajouri districts recorded the highest disease incidence 34.80% (30.00 to 39.00%) followed by temperate (Parts of Poonch district) and subtropical (Kathua and Jammu districts) which recorded almost similar incidence of 18.20 % (12.00 to 25.00%) and 17.60% (10.00 to 28.00%) respectively. Mean disease incidence was 34.80 per cent in intermediate zone indicating the seriousness of the problem in the area followed by temperate and subtropical zone which recorded almost similar percent disease incidence of 18.20 and 17.60, respectively. One bacterium and fungi belonging to four genera were isolated from the disease sample. Identity of *E. chrysanthemi* pv. *chrysanthemi*, *Fusarium* sp., *Aspergillus* sp., *Phytophthora* sp. and *Helminthosporium* sp.

Key words: Stalk rot, *E. chrysanthemi* pv. *chrysanthemi*, *Fusarium* sp., *Aspergillus* sp., *Phytophthora* sp., *Helminthosporium* sp.

Maize (*Zea mays* L.) occupies an important place among the cereal crops and ranks next to wheat, rice and sorghum in production and productivity. It occupies an area of 140 million hectares producing grain yield of 577 million metric tonnes in the world. In terms of acreage, India stands next to United States of America, Brazil, China and Mexico with the cultivation in an area of 61 m hectares with a production of 86 m tonnes (Food and Agriculture organization, 1996). In India, unlike western countries, maize is chiefly used as food for human consumption and only a small portion is used as green fodder, animal, poultry feed and industrial raw material. In Jammu and Kashmir, maize being the staple food of intermediate zone is grown approximately in 3 m ha with an acreage of 2 m ha in Jammu division. The district wise area in Jammu division under this crop in Udhampur, Doda, Rajouri, Jammu, Poonch and Kathua is 55.58, 48.19, 44.89, 2677, 23.67 and 15.89 thousand hectare respectively (Directorate Economic and statistics J&K, 1999).

In recent years, the average yield of maize is declined and this is accounted for biotic, abiotic and nutritional stresses (Ahamad *et al.*, 1998; Ahamad *et al.*, 2000; Ahamad, 2005; Ahamad, 2007; Ahamad, 2009, Ahamad, 2011, Ahamad, 2013). Among different stresses, biotic factors are the major constraints in the production and productivity of maize (Desai and Hedge, 1990). Due to the attack of several plant disease caused by fungi, bacteria, viruses and nematodes, yield are often reduced considerably (Rangarajan and Chakravati, 1969).

Bacterial stalk rot of maize caused by *E. carotovora* var. *zeae* Sabet is one of the most destructive disease in several maize growing regions of India. Most of the maize hybrids and composite released in the country as well as local varieties have been found highly susceptible to this nemesis (Lal and Saxena, 1978). There are many reports of losses caused by stalk rot disease in maize. The disease was extremely destructive on Single crosses growing in double cross production fields at Tarai state farm UP (15-22 %). So this problem was undertaken to understand the major causal organisms, inoculation techniques, diseases development and severity in the regions.

MATERIALS AND METHODS:

The present studies were necessitated to be undertaken for generating primary information on the relevant aspects of stalk rot complex of maize . This was carried out in the laboratory of Regional Agricultural Research Station, Rajouri-185131, SKUAST-Jammu during Kharif 2007 and 2008. During Kharif 2007, survey was conducted in major maize growing belts in subtropical, intermediate and temperate zones of Jammu division. The area selected under subtropical zone (upto 500 msl) includes Jammu, Kathua , intermediate zone (upto 1050 msl) comprised of parts of Rajouri and Poonch and temperate zone (above 1050 msl) parts of Poonch district. Ten farmers fields were selected from each zone and from each field spots containing 20 plants were selected at random to ascertain the incidence of stalk rot of maize during flowering period. The per cent incidence of stalk rot of maize was calculated using the formula as under:

$$\text{Incidence (\%)} = \frac{\text{Number of plants showing disease symptoms}}{\text{Total number of plants observed}} \times 100$$

Isolation, Purification and Maintenance of isolates : The affected maize plants showing stalk rot symptoms were collected and brought to laboratory . Small bits of disease stalk of 1 cm size of half healthy and half diseased portion were surface sterilized in 0.1 percent mercuric chloride (HgCl₂) solution for 2 minutes, washed 3 times in sterilized distilled water , then placed in Petriplates (9 cm diameter) containing Potato dextrose agar (PDA) medium. The fungi involved were incubated at 25± 1⁰C for 72 hours. The fungi involved were purified, and pure cultures were maintained in PDA slants for identification and inoculation. The bacterial isolates involved were further transferred from original Petri plates to ones having nutrient agar (NA) medium and pure culture were obtained (Ferreira- Pinto *et al.* 1994). The plates were incubated at 28± 1⁰C for 48 hours . The predominant colonies were selected and loopful of bacteria was streaked again on NA medium in Petri plates to obtain pure cultures. All bacterial isolates were maintained on NA slants at 5⁰C.

Identification : Identification of fungal organism associated with stalk rot of maize was attempted by observing the fungal cultures for their diagnostic character and by preparing their slides mounted in water, lactophenol and cotton blue. Colony characters, colour of the mycelium position of conidiophores and their shape were studied in accordance with description given in the literature for each organism (Barnet and Hunter (1972). Identification of bacteria associated with the disease was done by studying the morphological characters of colonies , host range, cultural characters and biochemical reactions. All isolates were tested for grams reaction . Colony characteristics were studied on NA and PDA after 48 hours of incubation at 28± 1⁰C. Potato soft rot tests were also conducted following the techniques of Ferrera-

Pinto *et al.* (1994). Acid production from organic compound was observed on peptone water (1g/l) with bromocresol purple as indicator. Sensitivity to erythromycin, reducing substances from sucrose were also determined (Schaad, 1988).

Inoculation techniques : Four different inoculation techniques viz., hypodermic syringe, root inoculation, leaf whorl and scissor leaf inoculation were evaluated during Kharif 2007,2008 to ascertain that best effective method for use in the day to day studies (Hingorani *et al.* 1959; Rangarajan and Chakravarti (1969) maize plants of Pioneer XOG-79 as susceptible cultivar were raised from surface sterilized seeds in pots containing sterilized field soil. Ten plants of one month age were used in each technique using bacterial cell suspension (2×10^8 cfu ml⁻¹) during Kharif 2007,2008.

i) **Hypodermic syringe inoculation :** 5 ml of actively growing culture suspension of bacterium was injected into the basal portion of stalk of maize plants by hypodermic syringe.

ii) **Root inoculation :** Plants were removed from pots washed with SDW thoroughly to remove all attached soil particles and root tips were excised by scalpel. The plants were then kept in a flask containing 25 ml of bacterial cell suspension to cover the injured root tips for 24 hours. The treated plants were replanted in pots containing autoclaved soil.

iii) **leaf whorl inoculation:** 15 ml of bacterial cell suspension was poured and placed in the leaf whorl of test plants.

iv) **Scissor leaf inoculation:** The leaves of each test plant were cut with scissors and the injured leaves were dipped in bacterial cell suspension for half an hour and then kept for observation.

For comparison same number of maize plants were given similar treatment in each techniques using sterilized water in places of bacterial inoculum. All the inoculated plants along with the checks were kept in locally manufactured moist chamber (pots kept in trays containing water and covered around with consequently wet muslin cloth in shade) for 48 hours before shifting them to glass house. Observations on infection development were recorded for two weeks at an interval of 2 days.

Pathogenicity: Pure culture of all the isolated organisms (five in number) were used for inoculating one month old plants of maize variety Pioneer XOG-79 with hypodermic syringe method in each case in glass house. Subsequently the observation for reproduction of stalk rot symptoms were made for each test organism upto 3 weeks.

RESULT AND DISCUSSION:

An extensive survey on prevalence of stalk rot of maize was undertaken at flowering stages in major maize growing areas covering 30 locations in four district of Jammu province (Table 1). A perusal of data revealed the occurrence of stalk rot complex in all the districts under three zones with variation in disease incidence. The intermediate zone covering Poonch and Rajouri districts recorded the highest mean disease incidence 34.80% (30.00 to 39.00%) followed by temperate (Parts of Poonch district) and subtropical (Kathua and Jammu district) which recorded almost similar incidence of 18.20% (12.00 to 25.00%) and 17.60% (10.00 to 28.00%) respectively. The overall mean disease incidence of stalk rot of maize during Kharif -2007,2008 were 23.53% with a range of 10.00 to 39.00%.

Identification of organisms and their percent frequency: The fungal and bacterial organisms isolated were identified by comparing their morphology and other characters with the details given in the relevant literature.

Fungal/ bacterial organisms: Four different genera of fungi and one bacteria were identified with the following diagnostic characters.

i) ***Fusarium* sp.:** Mycelium extensive and cottony in culture with pinkish in colour, conidiophores slender and simple, conidia hyaline, principally of two types macroconidia and microconidia. The macroconidia several celled, slightly curved or bent at the pointed ends. Microconidia ovoid or oblong.

ii) ***Phytophthora* sp. :** Sporangiohores compound, sympodial, slender, hyaline, branched and indeterminate in growth. Sporangia thin walled hyaline ellipsoid with conspicuous papillae at the apex.

iii) ***Aspergillus* sp.:** Conidiophores upright, simple terminating in a globose swelling, bearing phialides directly without metulae. The phialides radiating from the entire surface of the swelling. Conidia one celled globose in dry basipetal chains.

iv) ***Helminthosporium* sp.:** Mycelium dark in culture, stromata present, conidiophore single erect and simple. Conidiophores sympodium determinate in growth bearing a conidia at the apex. Conidia multiseptate, subhyaline to brown in colour

v) ***Erwinia chrysanthem* pv. *chrysanthemi* i:** The bacterium isolated from diseased tissue of maize plants exhibiting stalk rot symptoms was gram negative and single rod shaped. The colonies were visible after 48 hours on NA medium and were circular entire convex glistening and dirty white in colour. On potato dextrose agar medium colonies were cream glistening opaque circular, umbonate with undulating margins after 3-5 days of growth. All isolates produced soft rot on potato slices. All cultures produce reducing substance acid was produced from glucose and a zone of inhibition around the disc containing erythromycin was recorded (Table 2). All the above tests were positive for the confirmation / identification of *Erwinia chrysanthemi* pv. *chrysanthemi*.

Keeping in view the complex etiology of stalk rot of maize, representative samples 30 percent infection with incubation period of 10-11 days during Kharif 2007, 2008 whereas *Phytophthora* sp., *Aspergillus* sp. and *Helminthosporium* sp. failed to cause stalk rot of maize. Thus could not prove pathogenic.

Inoculation techniques and disease development : The data on comparative efficacy of different inoculation technique viz. Hypodermic syringe, root inoculation, leaf whorl and scissor leaves using four isolates of *E chrysanthemi* pv. *chrysanthemi* one each from the districts namely Jammu, Kathua, Rajouri and Poonch are presented in (Table 5) hypodermic syringe technique proved most effective giving 100 percent infection incidence with all the four isolate of *E chrysanthemi* pv. *chrysanthemi* followed by root inoculation technique which yield infection incidence ranging from 40 to 60 percent with different isolates. The incubation period of *Erwinia* stalk rot was also shorter (6-7 days) with hypodermic syringe than root inoculation technique which recorded incubation period of 9-10 days. Leaf whorl and scissor leaf inoculation methods failed to cause infection. Inoculated plants maintained in pots showed yellowing and wilting of the leaf with the initiation of stalk rot lesion on stalk. Leaves in top whorl particularly the younger ones started showing symptoms of wilting with conspicuous wet brown areas on leaf lamina starting from top downwards. The wilting progressed rapidly and plants completely wilted and dried within two weeks and even stalk of maize crop became unfit for fodder purpose at the time of harvesting. The progress of rotting in length and breadth was visible from 7th day of inoculation and progressed rapidly upto 13th day. Thereafter it remained constant and finally the plants died. The maximum length of rotted portion was recorded 3.5 cm and breadth 1.5 cm. Bad odour emanated from rotted portion of the stalk. The rotted stalk when split open showed discoloured soft rotted tissue.

The data revealed that the mean disease incidence was 34.80 % in intermediate zone indicating the seriousness of the problem in the area followed by temperate and subtropical zone which recorded almost similar per cent disease incidence of 18.20 and 17.60 %, respectively. One bacterium and fungi belonging to four genera were isolated from the disease sample. Identity of *E. chrysanthemi* pv. *chrysanthemi*, *Fusarium* sp., *Aspergillus* sp., *Phytophthora* sp. and *Helminthosporium* sp. Due to the complex etiology of the disease, the percentage frequency of each organism was calculated. *E. chrysanthemi* pv. *chrysanthemi* was most predominant encountered in all the districts with average frequency of 96.65% followed by *Fusarium*, *Aspergillus*, *Phytophthora* and *Helminthosporium* with 24.93 %, 13.60%, 11% and 6% respectively.

Pathogenicity tests indicated that only *E. chrysanthemi* pv. *chrysanthemi* and *Fusarium* sp. could cause the disease and remaining organism were non-pathogenic. Hypodermic syringe technique was proved the most effective out of the 4 techniques tested for artificial inoculation.

Present investigation have generated information on various aspects viz. prevalence of the disease in Temperature, Intermediate and Subtropical zones of Jammu division. Complex of organism/pathogens involved, inoculation techniques. The results obtained during the investigation are discussed in juxtaposition with finding of past workers. Thirty location of Jammu, Poonch, Rajouri and Kathua districts which were the most potential maize growing belts were surveyed and stalk rot of maize was encountered in all the areas. The incidence of disease varies in different districts/Zones, intermediate and temperate regions of Poonch and Rajouri district. The average incidence was highest ranging from 30.00 to 39.00 % followed by subtropical regions of Jammu and Kathua districts with 10.00 to 28.00%. The mean disease incidence of Jammu division was 23.53% (Table 1). Desai and hedge (1990) also reported as high as 38.60 and 36.50 per cent incidence of stalk rot of maize in Rabi and Kharif respectively from Karnataka. The incidence of stalk rot may vary from season to season and region to region depending upon biotic and abiotic factors. Survey and surveillance based on the incidence of plant disease is a prerequisite for assessing the extent of loss. This becomes most pertinent when percent incidence of any disease is directly related to the percent loss. In stalk rot of maize incidence during tussling and silking stage particularly bacterial stalk rot which corresponds to direct percent loss in yield as most of the bacterial stalk rot which corresponds to direct percent loss in yield as most of the bacterial stalk rot plants collapse and fall down to the ground without giving effective cobs. Hence, the incidence of disease worked out in the present survey amounts to the estimation of losses due to stalk rot of maize in maize growing areas of Jammu province.

Based on the laboratory examination of 57 numbers of maize stalk rot samples collected during kharif 2007-08 only four fungi *Fusarium* sp. *Phytophthora* sp. *Aspergillus* sp. *Helminthosporium* sp. and one bacterium *Erwinia chrysanthemi* pv. *chrysanthemi* were found associated with the disease. However, *E. chrysanthemi* pv. *chrysanthemi* was found most predominant occurring in all the four district with average frequency percentage of 96.65 followed by *Fusarium* sp. *Aspergillus* sp. *Phytophthora* sp. and *Helminthosporium* sp. with 24.93, 13.60, 11.60 and 8.96% respectively (Table 3).

The association of fungal and bacterial organisms with stalk rot of maize has been extensively reported by previous workers. Stalk rot of maize caused by *Erwinia* spp. has already been reported by many works (Reifschneider and Lopes 1982; Ferreria-

Pinto *et al.* 1994). The association of *Fusarium* and *Helminthosporium* was also previously reported (Bai *et al.*1988; Ramsey, 1990). Bacteria stalk rot of maize were monitored by Payak (1972), and Prasad and Sinha (1977). Whereas *Aspergillus* sp. and *Phytophthora* sp. have not been recorded earlier in association with stalk rot of maize. In pathogenicity (Table 4) even with hypodermic syringe inoculation method . The involvement of these organism in present investigation might be due to their secondary invasion at later stages of host plant .Thus out of the five organisms only two, *E.chrysanthemi* pv. *chrysanthemi* and *Fusarium* sp. were found pathogenic giving 100 % and 30% infection, respectively in artificially inoculated plants with hypodermic syringe method. Involvement of *E. chrysanthemi* , *Fusarium* sp. and *Hemlinthosporium* sp. have been investigated by above workers and no report for the involvement of *Aspergillus* and *Phytophthora* in stalk rot of maize was encountered in literature to the present findings.

Hypodermic syringe inoculation technique proved most effective giving 100 per cent infection in 6-7 days followed by root inoculation which gave 50 percent infection in 9-10 days while other two techniques failed to cause infection (Table 5) . These results are in accordance with the findings of Prasad and Sinha (1978), Rangarajan and Chokrovvari (1969), Kong and Lu (1994) who reported hypodermic syringe and root inoculation methods as most effective and convenient.

CONCLUSIONS: In the absence of availability of effective chemical control measure against bacterial stalk rot o maize , resistant varieties will form an important component of integrated management of the disease . Rigorous screening for resistance is a pre-requisite for an effective resistance breeding Programme.The incidence of disease varies in different districts/ Zones. In intermediate and temperate regions of Poonch and Rajouri district. The average incidence was highest ranging from 30.00 to 39.00 % followed by subtropical regions of Jammu and Kathua districts with 10.00 to 28.00 percent .

Table 1. Percent incidence of stalk rot of maize during Kharif 2007,-2008.

Zone	District	No. of locations	Mean disease incidence (%)	Range (%)
Zone I [*]	Jammu	5	15.20	10.00-28.00
	Kathua	5	20.00	14.00-24.00
	Zone mean		17.60	10.00-28.00
Zone II ^{**}	Rajouri	5	34.40	30.00-38.00
	Poonch	5	35.20	31.00-39.00
	Zone mean		34.80	30.00-39.00
Zone III ^{***}	Poonch Temperate	10	18.20	12.00-25.00
	Zone mean	-	18.2	12.00-25.00
			18.2	12.00-25.00

*Subtropical (upto500m)

** Intermediate (upto 1050)

*** Temperate (>1050m)

District	No. of sample	<i>Erwinia chrysanthemi</i>	<i>Fusarium</i> sp.	<i>Phytophthora</i> sp.	Aspergillus sp.	<i>Helminthosporium</i>
Jammu	11	100.00	27.27	0.00	9.09	09.09
Kathua	16	100.00	12.50	0.00	12.50	06.250
Poonch	14	92.85	28.57	21.42	14.28	14.28
Rajouri	16	93.75	31.25	25.00	18.75	06.25
Average	-	96.65	24.93	11.60	13.65	08.96

Table 2 Characteristics of strains of *E. chrysanthemi* isolated from maize

Test	Reaction
1.Potato soft rot	+
2. Reducing substances from sucrose	+
3.Sensitivity to everythromyci	
4.Acid production from organic compound	+

+ = Positive reaction

3 :District wise distribution of organism associated with stalk rot of maize during Kharif 2007. Frequency of occurrence (%)

Table 4: Pathogenicity of different organism associated with stalk rot of maize on a susceptible varietiy pioneer XOG-79

Organism	Percent plant infected *	Reaction **	Incubation period (Days)
<i>Erwinia chrysanthemi</i>	100.00	+	6-7
<i>Fusarium</i> sp.	30.00	+	9-10
<i>Phytophthora</i> sp.	0.00	-	-
Aspergillus sp.	0.00	-	-
<i>Helminthosporium</i> sp.	0.00	-	-

*Average of 10 plants

** Pathogenic

- Non pathogenic

Table 5 Comparative efficacy of different inoculation techniques for stalk rot of maize on Pionner XOG-79.

Inoculation Technique	Percent incidence				Incubation period (days)
	Isolates of Erwine chrysanthemi				
	E ₁	E ₂	E ₃	E ₄	
Hypodermic syringe	100.00	100.00	100.00	100.00	6-7

Roots	50.00	60.00	50.00	40.00	9-10
Leaf whorl	0.00	0.00	0.00	0.00	-
Scissor leaves	0.00	0.00	0.00	0.00	-

- on average of 10 plants inouolumn concentration 2×10^8 cfu^{-ml}
 E₁ E₂ E₃ E₄ represent isolate of *Erwinia chrysanthemi* from Jammu, Kathu , Rajouri and Poonch, respectively.

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