Comparison of three methods (leaf, soil and stem) of inoculation for stem rot jute (*Corchorus olitorius* L. and *C. capsularis* L.) caused by *Macrophomina phaseolina* (Tassi) Goid.

RAJIB KUMAR DE

J. Mycopathol, Res, 54(1) : 59-63, 2016;
ISSN 0971-3719

Department of Botany,
University of Calcutta,
Kolkata 700 019, India

J. Mycopathol. Res. ***54(1)*** *: 59-63, 2016;* (ISSN 0971-3719)

Comparison of three methods (leaf, soil and stem) of inoculation for stem rot jute (*Corchorus olitorius* L. and *C. capsularis* L.) caused by *Macrophomina phaseolina* (Tassi) Goid.

RAJIB KUMAR DE
Crop Protection Division, ICAR - Central Research Institute for Jute and Allied Fibres, Barrackpore, Kolkata 700 120

Received : 11.03.2015 RMS Accepted : 07.08.2015 Published : 25.04.2016

In order to devise a suitable method of inoculation of jute (*Corchorus olitorius* L. and *C. capsularis* L.) stem rot caused by *Macrophomina phaseolina* (Tassi) Goid. for large scale use, three methods (leaf, soil and stem) were compared. Among four leaf inoculation methods, tip cut and cotton swab method was better than inoculating floating leaf disc (8 mm), floating leaf and cotton swabbed detached leaf with typical symptom with light to dark brown spots in 100 % inoculated leaves within 48 hours on susceptible line JRC 412 which enlarged on further incubation. Among three different times of soil inoculation, 7 days before sowing was more effective in causing infection in JRO 524 and JRO 8432 with 26.6 and 23.3% stem rot than soil inoculated during sowing (15%) and 7 days after sowing (10%). Resistant reaction of four lines (OIN 125, OIN 154, OIN 651 and OIN 853) was confirmed by these three soil inoculation methods with JRO 524 as check. Stem inoculation technique was highly efficient and caused 100% infection with typical stem rot symptoms with brown spots of different length and intensity encircling the stem in JRO 524 compared to earlier methods. The variation in virulence pattern of isolates was evident, as more virulent isolate (from Sorbhog, Assam) produced distinctly clear, longer and darker brown stem rot lesion than less virulent ones (from Barrackpore, West Bengal).

Key words: Jute, *Corchorus* spp., Stem rot, *Macrophomina phaseolina,* Inoculation method

INTRODUCTION

Jute (*Corchorus olitorius* L. and *C. capsularis* L.) is also popularly called 'golden fibre'. It is grown as pre-*kharif* crop mainly in the eastern states of India mostly in West Bengal, Bihar and mostly in Assam contributing 77.0, 17.1 and 5.5% of National production, respectively (Anonymous, 2013). Popularity of jute has been declining nowadays among producers due to competition with cheap synthetics but jute would emerge soon with stronger positive attributes for its eco-friendliness with more oxygen producing, carbon dioxide absorbing and higher fuel wood producing capabilities, apart from its biodegradable diversified products. Stem rot caused by *Macrophomina phaseolina* (Tassi) Goid. is economically the most important disease affecting both yield and quality of fibre and seed in both cultivated species in all jute growing areas in India and other countries. On an average ten to fifteen percent yield loss due to stem rot has been esti-

Email : rkde@rediffmail.com

mated in different jute growing regions of India. Stem rot is the common name but the pathogen may infect any part of the plant at any stage of growth right from germination to harvest, attributing producing various symptoms, like, damping-off, seedling blight, leaf blight, stem rot, collar rot, root rot, stem break and spot on pod especially in seed crop (Roy *et al*, 2008).

Owing to its devastating nature, the disease causes nightmare for researchers and farmers as well. At times, this disease threatens both fibre and seed crops and is considered the major constraint in all the jute growing belts. Both the cultivated species of jute, i.e., *C. olitorius* and *C. capsularis* are equally affected by this disease showing no preference. Rainfall pattern, soil temperature and soil moisture determine the extent of damage. Stem rot is more prevalent in acid soil with pH below 5.8 and K deficiency. Seed yield also declines both quantitatively and qualitatively due to this disease. The disease is seed, soil as well as air borne and its management targets manipulation of soil, pre-sowing seed treatment and foliar spraying of fungicides or judicious combination of all.

Easy method of inoculation of jute stem rot for evaluation of various lines was not available in literature. However, sick plot method in hot spot location was reported by Mandal *et al*, (2000) and De and Mandal (2012 a, b).

In order to devise a suitable and efficient method of inoculation of jute stem rot for large scale use in rapid mass screening of germplasm against *M. phaseloina,* was carried out with the present study.

MATERIALS AND METHODS

All the three inoculation methods (leaf, soil and stem) were evaluated in 30 day old plants grown in pots (45 cm diameter) containing sterilized sandy loam soil (pH 6.5 - 7.5) during normal growing season of jute in April to July, 2012- 14 in CRIJAF, Barrackpore located at Nilganj, district North 24 Parganas, West Bengal. Inoculum was prepared aseptically by mycelial bit taken from a 3 day old freshly grown culture of *M. phaseolina* on potato dextrose broth at $28\pm1^{o}C$ for 5 days. The mycelial mat was filtered and used as inoculum for leaf or soil or stem inoculation. Inoculum density was 3.7×10^{2} colony forming units per g at the time of inoculation.

Leaf inoculation was done on both susceptible line JRC 412 and resistant line CIM 036 in the following four methods. In (1) leaf floating method, entire leaf was detached from plant and floated in Petri plate containing sterile water before inoculation. In (2) leaf swab method, detached leaf was given sterile cotton swab to maintain turgidity. Uniform leaf discs of 8 mm diameter were cut and floated in Petri plate containing sterile water before inoculation in (3) floating leaf disc method. In (4) leaf tip cut and swab method, a minor injury was made in leaf tip and kept for incubation giving wet sterile cotton swab. Above inoculum was used @ 2 g per leaf. Final observation (single) was noted in each of the methods when un-inoculated check showed clear and distinct difference from inoculated ones.Soil inoculation was done on plants grown in similar sized pots at three different times, namely, (5) 7 days before sowing, (6) during sowing time and (7) 7 days after sowing on two varieties, namely, JRO 524 and JRO 8432. Above inoculum was used @ 25 g per pot. (8) Stem inoculation technique comprised of making minor injury using sterile sand on stem of jute plants at 10 - 12 cm above soil just before inoculation with above pathogen. Two different isolates (isolated and purified from Sorbhog, Assam and Barrackpore, West Bengal) were used for inoculation separately. Above inoculum was used @ 2 g per stem.

RESULTS AND DISCUSSION

Leaf inoculation of M. phaseolina in jute

In leaf floating method and leaf swab method, neither any growth of the pathogen or nor symptom of stem rot was observed even after 96 hours of incubation. Similarly, in cotton swabbed detached leaf method, no growth or symptom of jute stem rot was noticed. In both cases, as there was no injury or wound on leaf tissue this fungal pathogen was unable to penetrate and cause infection on inoculated jute leaf. In floating leaf disc method, *M. phaseolina* grew vigourously and completely covering (100 % area) the top surface of disc within 96 hours. In leaf tip cut and swab method, typical symptom with light to dark brown spots were observed in 100 % inoculated leaves within 48 hours on susceptible variety JRC 412 which enlarged on further incubation (Table 1, Figure 1A). But no leaf symptom of jute stem rot was recorded in the leaves of resistant variety CIM 036 even after 120 hours of inoculation; it looked exactly similar to un-

Table 1 : Leaf inoculation of stem rot pathogen *Macrophomina phaseolina* on two jute lines JRC 412 and CIM 036

Method of inoculation	Inoculation on	Result*	Remarks*
Leaf floating method	Floating detached leaf	No growth or symptom	Unsuitable
Leaf swab method	Cotton swabbed detached leaf	No growth or symptom	Unsuitable
Floating leaf disc method	8 mm floating leaf disc	Vigourous growth	Suitable
Leaf tip cut and swab method	Injured leaf tip with cotton swab	Typical symptom with light to dark brown spots	It differentiated resistant (CIM 036) and susceptible (JRC 412) lines.

*Final (single) observation was noted in each of the methods when un-inoculated check showed clear and distinct difference from inoculated ones.

inoculated check without any inoculum. Injury might have played a role in entry and penetration of the pathogen. It caused infection successfully and finally produced typical symptom with light to dark brown rot. Among above four methods, the leaf tip cut and swab method was easy, quick and reproducible as it could differentiate disease reaction between symptoms in resistant (CIM 036) and susceptible (JRC 412) lines of jute.

Table 2 : Reactions of 4 + 2 entries to stem rot caused by *Macrophomina phaseolina in vitro* under three different times of soil inoculated conditions

Name of the entry	Inoculation 7 days before sowing on 09.05.2013		Inoculation during sowing on 16.05.2013		Inoculation 7 days after sowing on 23.05.2013		Remarks
	Number of plants inoculated	% of infected plants	Number of plants inoculated	% of infected plants	Number of plants inoculated	% of infected plants	
OIN 125	30	0	30	0	30	0	Resistant
OIN 154	30	0	30	0	30	0	Resistant
OIN 651	30	0	30	0	30	0	Resistant
OIN 853	30	0	30	0	30	0	Resistant
JRO 524	30	26.67 (31.06)	30	15 (22.75)	30	10 (18.31)	Susceptible
JRO 8432	30	23.33 (28.85)	30	15(22.75)	30	10 (18.31)	Susceptible
CD (P=0.05)	-	(1.55)	-	(1.33)	-	(2.26)	-
SEm±	-	(0.78)	-	(0.63)	-	(1.08)	-

*Figures in the parentheses represent angular conversion values.

Soil inoculation of M. phaseolina in jute

Among the three different times of soil inoculation with *M. phaseolina*, inoculation at 7 days before sowing was most effective in causing infection of stem rot in the susceptible checks as the added inoculum got extra time to establish and multiply in the soil. In both JRO 524 and JRO 8432, 26.6 and 23.3 % stem rot was observed, respectively, when soil was inoculated 7 days before sowing. But, only 15 % plant of both the varieties were infected with *M. phaseolina* when the soil were inoculated during sowing time. Inoculation done 7 days after sowing was the least effective method of inoculation of this fungus as it got very less time to establish and multiply in the soil and caused only 10 % stem rot infection in both JRO 524 and JRO 8432. Resistant reaction of four entries, namely, OIN 125, OIN 154, OIN 651 and OIN 853 was also confirmed by these three soil inoculation methods with variety JRO 524 as susceptible check. Out of 30 plants, typical symptom of stem rot was not observed in any of the plants of four resistant entries when soil was inoculated either 7 days before sowing or during sowing time or 7 days after sowing (Table 2, Figure 1B).

Table 3 : Stem inoculation technique of stem rot of jute *(Corchorus olitorius* L.) variety JRO 524 (Naveen) caused by *Macrophomina phaseolina* (Tassi) Goid.

Inoculation method	Barrackpore isolate		Sorbhog isolate		Remarks
	% stem rot	Colour of stem rot	% stem rot	Colour of stem rot	
Stem inoculation	32	Brown	54	Dark brown	Typical symptoms of stem rot
Check with sterile sand	0	-	0	-	No symptom
Check un-inoculated	0	-	0	-	No symptom

Stem inoculation of M. phaseolina in jute

Stem inoculation technique was highly efficient and caused 100% infection with typical stem rot symptoms with brown rotting spots of different length and intensity encircling the stem in jute plants of variety JRO 524. Stem inoculation with Barrackpore isolate of *M. phaseolina* resulted in 32% of the plants infection with symptom of brown stem rot whereas, Sorbhog isolate resulted in 54% of the plants infection with symptom of dark brown stem rot. The variation in virulence pattern of the isolates was also evident, as more virulent isolate (Figure 1C) (from Sorbhog, Assam) produced distinctly clear longer and darker brown stem rot lesion than less virulent (Figure 1D) ones (from Barrackpore, West Bengal). Minor injury might have played a role in facilitating entry and penetration of the pathogen in stem inoculation also. No symptom of stem rot was recorded in case of either check with sterile sand or un-inoculated check (Table 3, Figure 1E).

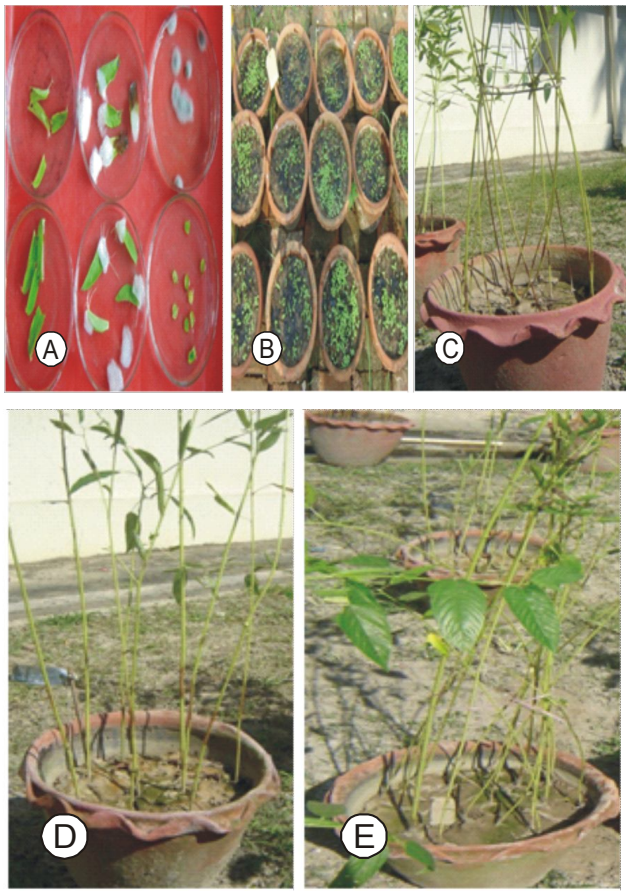

Fig. 1 : Comparison of leaf, soil and stem of inoculation for stem rot jute (*Corchorus* spp.) caused by *Macrophomina phaseolina* (Tassi) Goid. (A) leaf inoculation, leaf floating, leaf tip cut and swab, floating leaf disc, inoculated above and un-inoculated below; (B) soil inoculation; (C) stem inoculation with virulent isolate (Sorbhog, Assam), (D) stem inoculation with less virulent isolate (Barrackpore, West Bengal) and (E) un-inoculated check.

De and Mandal (2010, 2012a; b) reported that only four accessions (OIN 125, OIN 154, OIN 651 and OIN 853) out of 293 *Tossa* jute (*C. olitorius*) showed resistant reaction based on mean PDI 5.0 or less against stem rot after three years of evaluation in hot spot location at Sorbhog in district Barpeta of Assam and later their reaction was also confirmed in pot culture tests. Only six accessions (CIM 036, CIM 064, CIN 109, CIN 360, CIN 362 and CIN 386) out of 196 white jute (*C. capsularis*) showed their confirmed resistant reaction upon exposure to more vulnerable situation at Sorbhog (Mandal *et al,* 2000). Minor injury on leaf or stem tissue may be necessary to facilitate entry and penetration of the jute stem rot pathogen. Pectinolytic and cellulolytic enzymes play a significant role in the pathogenesis of seedling blight of jute caused by *M. phaseolina.* Both these enzymes were produced by *M. phaseolina* constitutively and inducibly. They were extracted from infected and surrounding regions. Distinct lesions were observed in 14 day old jute seedlings kept in enzyme solution at 21 ^{o}C for 48 hours (Chattopadhyay and Raj, 1978). Of the pectic enzymes contained in three strains of *M. phaseolina,* MP-C was found to be more virulent in causing disease and retting jute indicating a re-

lationship of pectic enzyme with stem rot disease and retting of jute (Myser Ali *et al*, 2005). De and Mandal (2007, 2008) developed a simple inoculation technique of *M. phaseolina* by leaf tip cut and wet cotton swab on resistant CIM 036 and susceptible JRC 412. To improve earlier technique of leaf inoculation, a new efficient stem inoculation method with 100 % successful infection was devised on variety JRO 524 grown in pots (De *et al*, 2014). De (2014) reported three different methods of inoculation of jute stem rot pathogen through leaf, stem and soil and found stem inoculation method to be the best.

Leaf inoculation (tip cut and swab) method may be applicable in the laboratory in a small scale for a quick testing or short sizing of large number of lines before massive field evaluation. Soil inoculation method at 7 days before sowing may be a useful tool for confirmation of disease reaction of few selected or elite lines after massive field screening. Among above three (leaf, soil and stem) methods, the stem inoculation technique was simple, easy and quick and has potential for use in identification of source of resistance in large scale screening of germplasm against *M. phaseolin* in the field or in pots in glass house.

ACKNOWLEDGEMENTS

The author is grateful to the Director, ICAR - Central Research Institute for Jute and Allied Fibres (Indian Council of Agricultural Research), Barrackpore, Kolkata 700120 for providing the necessary facilities for the experiments.

REFERENCES

Anonymous, 2013. Indian Jute. National Jute Board, 3A&3B Park Plaza, 71 Park Street, Ministry of Textiles, Government of India, Kolkata, India. December 2013, www.jute.com. XXV(2): 2.

Chattopadhyay, S. B., and Raj, S. K. 1978. Role of hydrolytic enzymes in seedling blight of jute incited by *Macrophomina phaseolina. Madras Agric. Journal* **65**, 320–324.

De, Rajib Kumar. 2014. Inoculation methods for development of stem rot of jute (*Corchorus spp*.) caused by *Macrophomina phaseolina* (Tassi) Goid. Paper presented in International Conference on Natural Fibres (Theme: Jute & Allied Fibres) held on August 1 - 3, 2014 at Grand hotel, Kolkata, India p 123 (OPA 32).

De, Rajib Kumar, H. Chowdhury and L. Chakraborty. 2014. New stem inoculation technique of stem rot of jute *(Corchorus olitorius* L. and *C. capsularis* L.) caused by *Macrophomina phaseolina* (Tassi) Goid. Paper presented in International Symposium on "Role of fungi and microbes in the 21st century – A global scenario" held on 20- 22 February, 2014 at Science City, Kolkata (Reference: www. imskolkata.org) pp 52.

De, Rajib Kumar and R. K. Mandal. 2007. Simple method of inoculation of stem rot pathogen *Macrophomina phaseolina* on jute (*Corchorus* spp.). Paper presented in the National symposium on Microbial biodiversity and plant health held at Farmers' Training Centre, BCKV, Lake Hall, Kalyani, West Bengal on 29-30 November, p , 38.

De, Rajib Kumar and R.K. Mandal. 2008. Simple method of inoculation of stem rot pathogen *Macrophomina phaseolina* on jute (*Corchorus* spp.). *Journal of Mycopathological Research* **46**: 138 1.13.

De, Rajib Kumar and R. K. Mandal. 2010. Identification of *tossa* jute (*Corchorus oliotorius* L) germplasm tolerant to stem rot pathogen *Macrophomina phaseolina* (Tassi) Goid. In Jute And Allied Fibres Production, Utilization And Marketing, Editors, Palit, P.; Sinha, M. K.; Meshram, J.H.; Mitra, S.; Laha, S. K.; Saha, A. R. and Mahapatra, B. S., Indian Fibre Society, Eastern region, Central Research Institute for Jute and Allied Fibres (CRIJAF), Barrackpore, Kolkata 700120. pp 266-270.

De, Rajib Kumar and R.K. Mandal. 2012a. Four *Corchorus olitorius* L. lines resistant to stem rot pathogen *Macrophomina phaseolina* (Tassi) Goid. Paper presented in the National Symposium on "Frontlines of microbiological research: concepts and applications" organized by Indian Mycological Institute, Kolkata, held at Department of Botany, University of Calcutta, Ballygunge Science College, 35, Ballygunge Circular Road, Kolkata 700 019, India on 02- 04 February, 2012 (Reference: www. imskolkata.org). pp. 21.

De, Rajib Kumar and R. K. Mandal. 2012b. Identification of resistant sources of jute (*Corchorus olitorius* L.*)* against stem rot caused by *Macrophomina phaseolina* (Tassi) Goid. *Journal of Mycopathological Research* **50:** 217- 222.

Mandal, R. K., Sarkar, S., and Saha, M. N. 2000. Field evaluation of white jute (*Corchorus capsularis* L.) germplasm against *Macrophomina phaseolina* (Tassi) Goid under Sorbhog condition. *Environment and Ecology* **18**: 814-818.

Myser Ali, M., Sayem, A. Z. M., Alam. S., and Ishaque, M. 2005. Relationship of pectic enzyme of *Macrophomina phaseoli* with stem-rot disease and retting of jute. *Mycopathologia* **38**: 289 – 298.

Roy, A., De, Rajib Kumar and Ghosh, S. K. 2008. Diseases of bast fibre crops and their management. In: Jute and Allied Fibre Updates, Central Research Institute for Jute and Allied Fibres, Barrackpore, Kolkata 700 120, West Bengal, India pp. 217 – 241.