Method Article

Optimizing Inoculation Techniques for Enhanced Rust Disease Pressure in Fig Trees: Insights on *Cerotelium fici* Multiplication and Maintenance

ABSTRACT:

Fig rust is one of the most prevalent diseases caused by *Cerotelium fici* is an obligate parasite and it has to be maintained only on its own host. The present study was taken into consideration to check the comparative effectiveness of various inoculation methods for inducing disease symptoms, maintaining and multiplying inoculums, and examining the slow rusting mechanism. For this, a polyhouse experiment using the Poona fig cultivar was conducted at the National Agricultural Research Project, Ganeshkhind, Pune. Ninety-days-old three fig layers, each with four to five healthy leaves, were inoculated in the evening using six different inoculation methods. The suspension of urediospores used for inoculation was made with 5 X 10^8 spores ml^{-1} in sterile water. The result of the study revealed that, leaf dip, micro droplet and hand inoculation methods were found most effective for mass multiplication and maintenance of *C. fici*

Keywords: Fig disease, rust, inoculation techniques, spore dusting, disease expression

INTRODUCTION:

Ficus carica L., often known as the common fig, is a prominent member of the Moraceae family and has long been prized for its delicious fruits. This species of plant is native to southwest Asia and the Mediterranean. It is considered temperate. Nearly everywhere in the world, the plant is grown commercially for its dried and fresh fruits (Solomon et al, 2006; Sharma et al, 2019). Although the fruits of other Ficus species can also be eaten, they are usually only eaten in the vicinity or used as bush food. The plant may have medical (Gousia et al, 2018) and religious significance (Sharma et al, 2019) in addition to its food benefits.

In Maharashtra fig is grown in Pune, Ahmednagar and Aurangabad districts where in Pune is the leading producer of fig with an area of 1052 ha with production of about 7373 metric tons and productivity of 7.0 tons/ha/year (Anonymous, 2011). Among the several constraints for low productivity of fig the prevalence of number of destructive diseases at different stages of its growth and development. Numerous diseases can severely impair the overall growth and yield of fig plants. The most common fungi diseases seen on fig plants are canker, fruit rot, internal rot, leaf spot, limb blight, rust, and soft rot (Michailides, 2003). Rust is one of the most prevalent fig and other major crop diseases that can have an impact on plant production and general growth (Khaire and Hake, 2028; Vyavhare et al, 2020; Khaire et al, 2020). It mostly affects the fig plant's leaves, with little to no harm to the fruits. Although fig trees frequently experience defoliation, plants are rarely killed by it (Latinovic

et al, 2015). Initially, tiny yellow spots develop on underside of leaves. They enlarge to form reddish brown angular spots visible on lower and upper leaf surfaces. Leaves are the mainly plant part affected severely by rust. However, under congenial condition symptoms also appear on petiole, shoots and fruits. Severe disease incidence results in to yellowing and defoliation. At present, the disease has become a major limiting factor in fig cultivation. The rust is an endemic disease and severe in all parts of the state where the crop is grown. The fig growers are much worried about the worst situation caused by this disease. About 50 per cent losses have been reported by earlier workers (Gaikwad and Nimbalkar, 2004 and Anonymous, 2022) indicating the seriousness of rust disease in fig. The present study was undertaken to find out comparative efficacy of different inoculation techniques to create high disease pressure, maintenance and multiplication of inoculums and also to study slow rusting mechanism.

Material and Methods

Standardization of inoculation methods for *C. fici*

For this, the experiment was carried out in polyhouse at National Agricultural Research Project, Ganeshkhind, Pune on Poona fig cultivar. Ninety days old three fig layers having 4-5 healthy leaves each were inoculated in the evening hours by different methods as detailed below. The urediospores suspension used for inoculation was prepared in sterilized water containing 5 X 10^8 spores ml^{-1}.

Micro droplet inoculation technique (MDIT): The inoculation was done as per methodology described by Munaut et al. (1997).

Spore dusting: Leaves of fig plant to be inoculated were first moistened by sprinkling distilled water and then fresh urediospores were dusted on them by tapping infected leaves on them (Fig 1f).

Stapler method: The uniformly rusted leaves of Poona fig were collected from the field and cut in to 2 cm X 2 cm pieces. These pieces were stapled on moistened healthy fig leaves of three-month-old -plants in such a way that the ventral surface of the diseased leaf touching to the ventral as well as dorsal surface separately (Fig 1e).

Leaf dip inoculation: The healthy leaves of fig plant grown in polyhouse were dipped in spore suspension of *C. fici* so that urediospores adhere to the leaves (Fig 1c).

Cotton swabbing: Absorbant cotton plug having the size of 2 cm diameter was dipped in urediospores suspension (5 X 10^8 spores ml^{-1}) and then swabbed gently on both the surfaces of healthy leaves of fig (Fig 1d).

Hand inoculation: The healthy leaves of fig plant grown in polyhouse were hand inoculated by freshly prepared spore suspension on the both sides of leaves so that urediospores adhere to leaf surfaces (Fig 1b).

Control: An uninoculated set of fig plants was maintained by spraying distilled water.

RESULTS:

Standardization of inoculation methods for *C. fici*

Different methods of inoculation with urediospores of *C. fici* were evaluated to find out most effective method for production of maximum disease in order to obtain fresh mass of urediospores for using in different pathological studies under artificial epiphytotic. The data in Table 1 reveal that all the methods of inoculation were effective in inducing the rust severity more than 50 per cent. However, among the seven methods of inoculation, leaf dip, micro droplet and hand inoculations were found most effective over all other methods wherein, significantly maximum (73.33 %) rust severity was observed with latent period of seven days. The next method in order of superiority was spore dusting inoculation (66.67 %) that was at par with above mentioned three methods and had a latent period of 7 days. It was followed by stapler method of inoculation, where the latent period was seven days with Per cent Disease Intensity of 60 per cent. Among all the methods, cotton swabbing showed more latent period (9 days) with least Per cent Disease Intensity of 53.33 per cent. Severity of rust produced by different methods of inoculations is illustrated in fig.1. Therefore, for all *in vivo* (pot culture) pathological trials, the simple and effective method, *i.e*. micro droplet inoculation was used.

Table 1: Intensity/severity of rust caused by *C. fici* in fig as influenced by different methods of inoculation

Sr. No.	Methods of inoculation	Latent period (Days)	PDI days after inoculation									
			6	7	8	9	10	11	12	13	14	15
1	Hand Inoculation	7	0.00 **0.00**	2.33 **9.72**	8.33 **16.59**	8.33 **16.59**	16.67 **23.84**	20.00 **26.55**	40.00 **39.22**	53.33 **46.90**	66.67 **54.97**	73.33 **59.19**
2	Dipping leaves in spore suspension	7	0.00 **0.00**	5.00 **12.92**	8.33 **16.59**	16.67 **23.84**	16.67 **23.84**	30.00 **32.29**	53.33 **46.94**	60.00 **50.75**	66.67 **54.97**	73.33 **59.19**
3	Micro droplet inoculation	7	0.00 **0.00**	2.33 **9.72**	6.67 **14.75**	16.67 **23.84**	16.67 **23.84**	23.33 **28.07**	33.33 **35.00**	53.33 **46.90**	60.00 **51.12**	73.33 **59.19**
4	Cotton swabbing	9	0.00 **0.00**	0.00 **0.00**	0.00 **0.00**	2.33 **9.72**	4.00 **8.93**	6.67 **14.75**	16.67 **23.84**	20.00 **26.55**	33.33 **35.00**	53.33 **46.90**
5	Stapler method I	7	0.00 **0.00**	2.33 **9.72**	6.67 **14.75**	8.33 **16.59**	15.00 **16.59**	16.67 **23.84**	33.33 **35.00**	46.67 **43.06**	60.00 **51.12**	60.00 **51.12**
6	Stapler method II	7	0.00 **0.00**	2.33 **9.72**	6.67 **14.75**	8.33 **16.59**	15.00 **16.59**	16.67 **23.84**	33.33 **35.00**	46.67 **43.06**	60.00 **51.12**	60.00 **51.12**
7	Spore dusting	7	0.00 **0.00**	2.33 **9.72**	7.33 **14.99**	16.67 **23.84**	16.67 **23.84**	33.33 **35.00**	40.00 **38.84**	46.67 **43.06**	53.33 **46.90**	66.67 **54.97**
8	water spray	--	0.00 **0.00**	0.00 **0.00**	0.00 **0.00**	0.00 **0.00**	0.00 **0.00**	0.00 **0.00**	0.00 **0.00**	0.00 **0.00**	0.00 **0.00**	0.00 **0.00**
	SE ±			**1.32**	**1.65**	**1.18**	**1.42**	**2.80**	**2.47**	**2.35**	**2.91**	**2.88**
	CD (0.05)			**4.04**	**5.06**	**3.63**	**4.35**	**8.57**	**7.56**	**7.21**	**8.91**	**8.81**
	CV%			**35.40**	**30.65**	**12.68**	**13.13**	**21.04**	**13.47**	**10.86**	**11.68**	**10.43**

Figures in bold faces are arc sin values

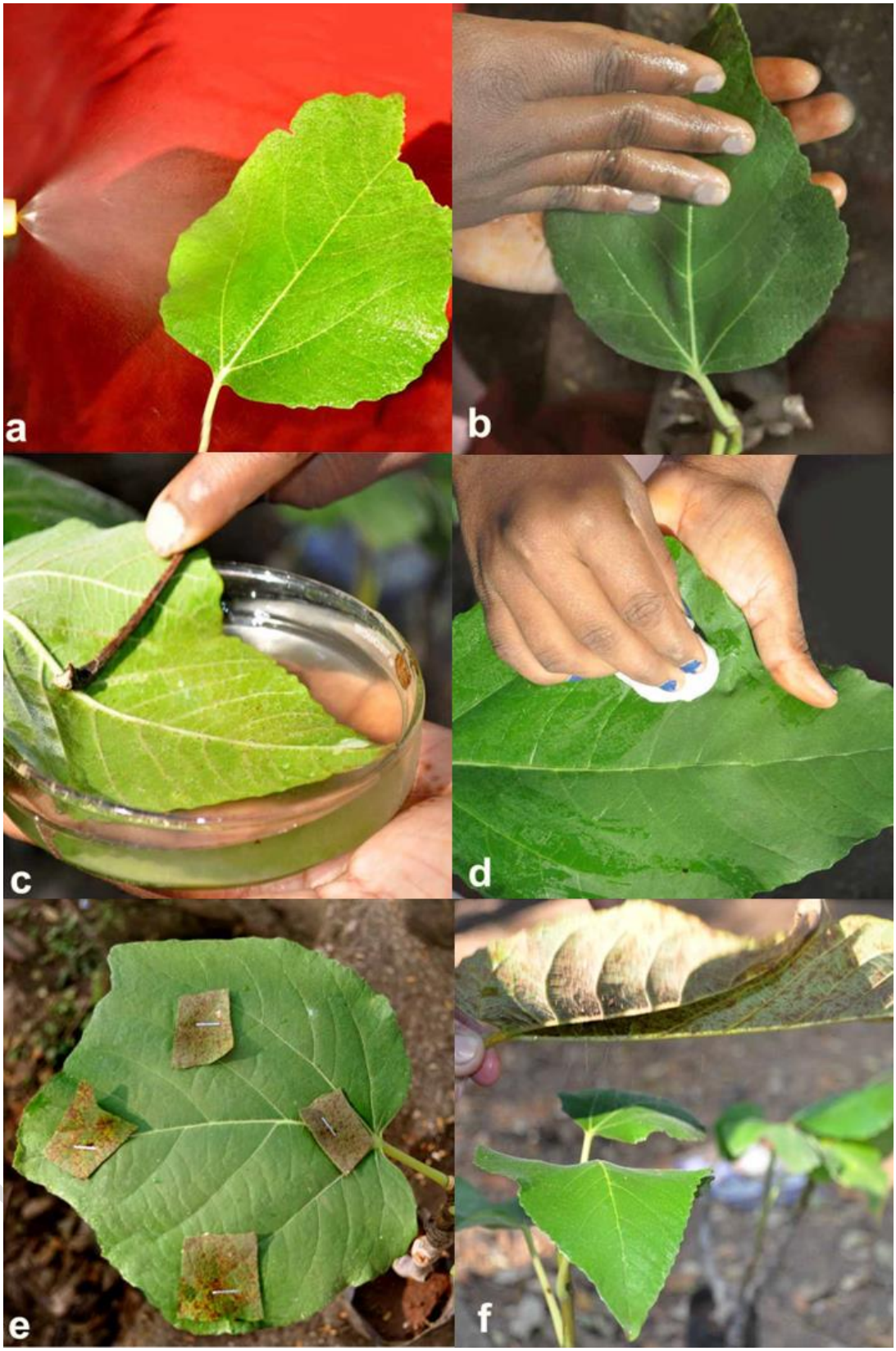

Figure 1: Different inoculation methods to produce high disease pressure, maintain and multiply inoculums for rust of fig incited by *Cerotelium fici* a) MDIT, b) Hand inoculation of spore suspension, c) Dipping leaves in spore suspension, d) Cotton swab invocation, e) Stapler method and e) Spore dusting

DISCUSSION:

Maintenance and multiplication of inoculum of the pathogen is essential for various studies. Since the *C. fici* is an obligate parasite, it has to be maintained only on its own host. Hence, different methods of inoculation with urediospores of *C. fici* were evaluated to find out most effective method for production of maximum disease to obtain fresh mass of urediospores for using in different pathological studies under artificial epiphytotics. In present investigation, all the tested methods of inoculation were found effective in inducing the rust severity more than 50 per cent. However, among the different methods of inoculation, leaf dip, micro droplet and hand inoculations were found most effective (73.33% severity) for mass multiplication of *C. fici*. The present results are inconformity with the Singh and Thapliyal (1977) and Chirme *et al.* (1997) who suggested spraying of inoculum as a convenient method for large scale maintenance and multiplication of *P*. *pachyrhizi* causing rust of soybean. In addition, efficient infection of *P. pachyrhizi* was obtained through the spore dusting method on soybean leaves by some scientists (Anonymous, 1971 and Singh and Thapliyal, 1977) that is in accordance with present results where spore dusting method was also observed to be better (66.67 % severity) for production of good amount of rust in fig. Further, Stapler method of inoculation was also found to be successful (60.0% severity) for multiplication of rust inoculums that is in agreement with Dadke (1996) who observed that stapler method was best for maintenance and multiplication of *P*. *pachyrhizi*. The forecited investigations divulge that micro droplet method of inoculation has practical utility for mass multiplication and maintenance of fig rust inoculum for further studies.

CONCLUSION:

It is concluded that, the leaf dip, micro droplet and hand inoculation methods were found most effective for mass multiplication and maintenance of rust inoculum.

REFERENCES

Anonymous. 2022. Area and production of fruits in Maharashtra. Directorate of Horticulture, Maharashtra state, Pune.

Anonymous. 2022. Chemical control of fig rust. Joint Agresco Report. 2004. RFRS, Ganeshkhind, Pune.

Chirme, B. B., Wuike, R. V., Patil, V. S. and Thakare, C. S. 1997. Efficacy of inoculation methods of Phakopsora pachyrhizi Syd. on soybean. J. Maharashtra Agric. Univ. 22: 262-263.

Dadke, M. S. 1996. Studies on rust of soybean [*Glycine max* (L) Merill] caused by Phakopsora pachyrhizi Syd. M.Sc (Agri.) Thesis, University of Agricultural Sciences, Dharwad.

Gaikwad, A. P. and Nimbalkar, C. A. 2004. Management of Fig rust with fungicides. *J. Mycol. Plant Pathol*. 34 (2): 418-419.

Gousia Gani1, Tabasum Fatima, Tahiya Qadri, Beenish, Nusrat Jan, Omar Bashir (2018) Phytochemistry and pharmacological activities of fig (Ficus carica): A review. International Journal of Research in Pharmacy and Pharmaceutical Sciences. 3 (2) 80-82.

Khaire P. B and Hake L. G. 2018. Disease Management of Kharif Green Gram and Black Gram. Popular Kheti. Vol 6: Issue 2. p 96-103.

Khaire Pravin, Mane Someshree and Uikey Darshana (2020) Management of Three Rust Diseases of Wheat Caused by *Puccinia* Pathogen. KRISHI SCIENCE – eMagazine for Agricultural Sciences Volume:01 Issue:02 – August 2020

Latinovic J., Radisek S. and Latinovic N., Severe Infection of Figs by Fig Rust Pathogen *Cerotelium Fici* in Montenegro *Agric. For*., 2015; 61(2): 101–107. DOI 10.17707/ AgricultForest.61.2.08.

Michailides, T. J. (2003). Diseases of fig. Diseases of Tropical Fruit Crops, 253-273. https://doi.org/10.1079/9780851993904.0253

Munaut, F., Hamaide, N. and Maraite, H. 1997. A standardized microdroplet inoculation technique for analysis of the interaction between *C. gloeosporioides* and *Stylosanthes guianensis*. Parasitica. 53 (4): pp:117-125.

Sharma V., Mishra S., Yesudas R. and Rajput R.S., (2019) A Review on *Ficus religiosa* (Sacred Fig). *International Journal of Research and Analytical Reviews* 6 (2) 901-906.

Singh, K. P. and Thapliyal, P. N. 1977. Some studies on the soybean rust caused by *Phakopsora pachyrrhizi. Indian J. Mycol. Pl. Pathol.* 7(1): 27-31.

Solomon A., Golubowicz S., Yablowicz Z., Grossman S., Bergman M., Gottlieb H.E., Flaishman M.A. Antioxidant activities and anthocyanin content of fresh fruits of common fig (*Ficus carica* L.) *J. Agric. Food Chem.* 2006;54:7717–7723. doi: 10.1021/jf060497h.

Vyavhare, Y.V., Mane, S. S., Khaire, P.B., and Joshi, M. S. 2020. Epidemiological studies on foliar diseases of groundnut in Konkan region. *Journal of Pharmacognosy and Phytochemistry*; Sp 9(5): 577-581

Anonymous. 2011. Area and production of fruits in Maharashtra. Directorate of Horticulture, Maharashtra state, Pune.

Anonymous. 1971. Annual Report. Taiwan Agriculture Research Institue, pp. 39-41.