Indian J. mycol. Res., 4 (1 & 2), 22—31 (1966).

PHYSIOLOGICAL STUDIES ON INCITANTS OF GUAVA WILT *FUSARIUM SOLANI* (MART.) APP. AND WR. EMEND SNYDER AND HANSEN AND *MACROPHOMINA PHASEOLI* (MAUBL.) ASHBY

By

S. B. CHATTOPADHYAY and S. K. BHATTACHARYYA*

Faculty of Agriculture, University of Kalyani, West Bengal

Studies were made of *Fusarium solani* and *Macrophomina phaseoli* the incitants of wilt disease of guava in West Bengal, to find out effect of different factors on growth and reproduction. *Richard's synthetic agar* was found best for growth of *F. solani* and *potato-dextrose-agar* for *M. phaseoli*. Optimum concentration of carbon for growth and sporulation of *F. solani* was between 3.0–4.0% and for *M. phaseoli* between 5.5–6.0%. Starch was the best source of carbon for *F. solani* and in *M. phaseoli*. Good growth was noted in all the cases except mannitol and glycerine. The optimum concentration fo nitrogen for *F. solani* was between 0.5% to 1.0% of KNO^3 below or above which there was slight reduction in growth. In case *M. phaseoli* with progressive increase in nitrogen concentration there was practically no increase in vegetative growth below 2%. Growth of *F. solani* and *M. phaseoli* decreased when the concentration of Indol-3-acetic acid was increased to 50 PPM. Sporulation of *F. solani* was completely suppressed at this concentration. In case of Napthal-acetic acid with increasing concentration there was progressive decrease in case of *F. solani*. But in case of *M. phaseoli* there was increase in vegetative growth with increasing concentration of Napthal-acetic acid. Optimum *p*H for *F. solani* and *M. phaseoli* was 6.0. Optimum temperature for growth of and sporulation of *F. solani* was between 25°–30°C. and for *M. phaseoli* 35°C. for growth, and 25°C. for sclerotia formation. Thermal-death-point of *F. solani* was approximately 60°C. and that for *M. phaseoli* 65°C. The optimum soil moisture for *F. solani* was 60% saturation and for *M. phaseoli* was 40%. With increase or decrease in the moisture content, growth of both the fungi was reduced.

Introduction

Guava plants in West Bengal suffer from a wilt disease incited by pathogens, *Fusarium solani* (Mart.) App. and Hansen and *Macrophomina phaseoli* (Manbl.) Ashby either alone or in combination (Chattopadhyay and Sen Gupta, 1955). As two organisms are involved and the same disease occurs in two widely different localities with different soil types, namely, the gangetic alluvium and leached red laterite soil, studies on physiology of these two fungi were taken up to have knowledge of the effect of various factors on growth and reproduction of these two fungi.

* Present address : Pathologist, Central Potato Research Instiutute, Patna.

Material and Methods

Fusarium solani Isolate 'A' and *Macrophomina phaseoli* isolated from wilted guava plants were used in the experiment. Single macroconidial cultures were used for *F. solani*. Cultures of *M. phaseoli* were purified by hyphal tip method. Stock cultures were kept on *potato-dextrose-agar* slants at 15°C. and were subcultured once a month.

5 mm. circular discs aseptically taken from 3-days-old culture grown on *potato-dextrose-agar* in Petri dishes were used for inoculating different media. Data were taken of linear growth of the fungi on solid media at various intervals and dry wt. of mycelium in several liquid media after 9 days of growth. The spore count of *F. solani* was taken with the help of haemocytometer slides. In case of *M. phaseoli* prepondereance of selerotia was noted after 9 days of growth and recorded in the following way : (+++) abundant, (++) moderate, (+) poor, and (—) nil. In each case a replicate of three was used. For studying the effect of soil moisture on the growth of these organisms in the laboratory, Norton's (1953) method was followed.

The *soil-extract-medium* of the followng composition was used: Agar agar, 12.5 gms.; Glucose, 1.0 gm.; Dipotassium phosphate, 0.5 gm.; Soil extract (Stock), 100 c.cm.; and Tap water, 900 cm. Stock solution of soil extract was prepared by mixing 1000 gms. of garden soil with 1000 c.cm. of tap water and then heating the same in the autoclave for 30 minutes. A small amount of calcium carbonate was then added and the solution filtered till the filtrate was clear.

Results

I. *Effect of Nutrients on Growth and Sporulation*

(a) *Different media :* Data on effect of different media (solid and liquid) on growth and reproduction of the two fungi at 25°C. are presented in Table 1. In *F. solani* the best linear growth was obtained in *Richard's synthetic agar*, highest dry wt. of the mycelium in *Brown's medium*, then in *Richard's medium*, and highest sporulation in *ammonium nitrate-sucrose-agar*. In case of *M. phaseoli* maximum linear growth was obtained in *potato-dextrose agar*, maximum dry wt. of the mycelium in *ammonium nitrate-sucrose liquid medium*, and highest number of sclerotia in *Czapek-Dox medium* with 0.32% malt extract.

(b) *Effect of different concentrations and sources of carbon compounds :* Carbon content of Richard's medium (solid and liquid) was varied from 1.0% to 7.0% to find out optimum carbohydrate concentration for growth and reproduction. To study the effect of different carbon sources sucrose glucose, maltose, starch, mannitol and glycerine were used instead of sucrose and starch in combination which constitute the sources of carbon in *Richard's medium*. Data are presented in Tables 2 and 3.

Table 1. *Growth and sporulation of* F. solani *and* M. phaseoli *in different media at 25°C.*

Organisms	Different Media								
	Potato Dextose	Czapek-Dox modified with 0.32% malt	Soil-extract	Peptone-glucose	Aspara-gin-glucose	Ammo-nium-nitrate-sucrose	Brown's	Czapek's	Ri-chard's
F. salani									
Linear growth in 8 days (mm.)	70	86	63	53	51	58	63	87	95
Dry wt. of mycelium (mg.)	29	17	3	23	12	30	40	29	39
Sporulation (million)	3,000	2,400	—	440	1,500	3,200	1,160	2,000	1,200
M. phaseoli									
Linear growth in 4 days (mm.)	88	70	34	44	52	68	52	55	72
Dry. wt. of mycelium (mg.)	44	42	14	27	8	54	12	34	40
Sclerotia	+++	+++	+	+++	+++	+++	++	++	++

Table 2. *Effect of different concentration of Carbon on growth and sporulation of* F. solani *and* M. phaseoli *in Richard's medium at* pH *6.0 and 25°C.*

Carbon concentrations	F. SOLANI		Sporulation (million)	M. PHASEOLI		
	Linear growth in 3 days (mm.)	Dry wt. of mycelium (mg.)		Linear growth in 3 days (mm.)	Dry wt. of mycelium (mg.)	Sclerotia
1.0%	88	7	11.8	90	24	+
2.0%	74	16	14.5	91	42	+
3.0%	93	14	16.0	85	42	+
3.5%	87	26	17.3	88	53	+
4.0%	83	29	19.5	85	64	+
4.5%	81	22	14.8	87	66	+
5.0%	76	20	14.0	100	66	++
5.5%	78	21	14.2	100	69	++
6.0%	81	20	15.0	94	81	++
6.5%	82	21	14.8	89	72	++
7.0%	84	23	14.3	83	78	++

Table 3. *Effect of different sources of Carbon on* F. solani *and* M. phaseoli *in Richard's medium at pH 6.0 at 25°C.*

Sources of carbon	F. SOLANI			M. PHASEOLI		
	Linear growth in 6 days (mm.)	Dry wt. of mycelium (mg.)	Sporulation (million)	Linear growth in 6 days (mm.)	Dry wt. of mycelium (mg.)	Sclerotia
Sucrose	78	28	8.8	100	69	++
Dextrose	85	22	10.6	100	64	++
Maltose	82	47	5.5	100	78	++
Starch	100	53	12.3	100	63	+++
Mannitol	72	30	7.9	32	12	—
Glycerine	64	38	6.8	100	19	—

From the data it would be seen that *F. solani* shows optimum growth and sporulation between 3% and 4% concentrations of carbon compounds. With further rise at 4.5% concentration there is reduction in growth and sporulation, above which there is parctically no further reduction.

In case of *M. phaseoli*, there is progressive increase in vegetative growth with increasing concentrations of carbon upto 6% after which there is slight reduction in growth. Higher concentrations, 5% and above, tend to increase sclerotial formation.

In *F. solani*, best growth and sporulation was obtained with starch. Next comes maltose in respect of growth, but sporulation was reduced. Mannitol and glycerine, did not appear to be good sources of carbon for growth.

(c) *Different concentrations of nitrogen compounds :* To find suitable concentration of nitrogen compounds for growth of *F. solani* and *M. phaseoli*, nine different concentrations of nitrogen in the form of KNO_3 in *Richard's medium* were used. The data are recorded in Table 4.

Table 4. *Effect of different concentfations of nitrogen (KNO_3) of* F. solani *and* M. phaseoli *on Richard's medium at pH 6.0 and 26°C.*

Nitrogen concentration	F. SOLANI		M. PHASEOLI	
	Linear growth in 6 days (mm.)	Dry wt. of mycelium (mg.)	Linear grotwh in 6 days (mm.)	Dry. wt. of mycelium (mg.)
0.25%	93	22	80	10
0.50%	81	32	65	11
0.75%	88	32	68	13
1.00%	83	31	70	12
1.25%	93	25	77	12
1.50%	87	25	83	10
1.75%	89	26	74	12
2.00%	85	20	64	19

Data in Table 4 show that growth of *F. solani* was optimum between 0.5% and 1.0%, below or above which there was slight reduction in growth. In *M. phaseoli*, with progressive increase in nitrogen concentration, there was practically no increase in vegetative growth until at the highest level used (2%) the mycelial production was augmented.

II. *Effect of Hormones of growth and Sporulation of* F. solani *and* M. phaseoli

To find out the effect of two synthetic hormones on growth and sporulation of *F. solani* and *M. phaseoli* indol-3-acetic acid and napthal-acetic acid were used in three different concentrations, namely, 1 PPM, 10 PPM and 50 PPM. One set was also kept as control. The growth, sporulation and selerotial formation were recorded in the usual way. The observations are presented in Table 5.

Table 5. *Effect of hormones on growth and sporulation of* F. solani *and* M. phaseoli *at 25°C.*

Hormones with concentrations	F. SOLANI			M. PHASEOLI		
	Linear growth in 8 days (mm.)	Dry wt. of mycelium (mg.)	Sporulation (million)	Linear growth in 8 days (mm.)	Dry wt. of mycelium (mg.)	Sclerotia
Indol-3-acetic acid						
1 PPM	100	36	1.2	100	102	+++
10 PPM	100	41	2.23	100	91	+++
50 PPM	68	30	—	100	86	+++
Napthal-acetic acid						
1 PPM	100	48	1.2	100	75	+++
10 PPM	100	43	0.02	100	94	+++
50 PPM	70	38	0.08	100	105	+++
Control	100	33	0.25	100	78	+++

Data in Table 5 show that in *F. solani* addition of indol-3-acetic acid at 1 and 10 PPM vegetative growth and sporulation were increased, but at 50 PPM growth was reduced and sporulation was completely supressed. Addition of napthal-acetic acid increased vegetabtive growth but with increasing concentration, there was reduction in mycelium. Sporulation was adversely affected at 10 and 50 PPM. In *M. phaseoli* with addition of Indol-3-acetic acid at 1 PPM there was reduction, but in all cases showed better results in comparison with check. With Napthal-acetic acid, there was progressive increase in vegetative growth with increasing concentration. Sclerotial formation was not affected in either case at any concentration.

III. *Effect of* pH *on growth and sporulation of* F. solani *and* M. phaseoli

To find the effect of different levels of *p*H on growth and sporulation of the two organisms *Richard's liquid medium*, was adjusted to different

*p*H by N lactic acid and N. NaOH. The organisms were inoculated and allowed to grow for 9 days at 22°C. Data of the experiment are presented in Table 6.

Table 6. *Effect of p*H *on growth and sporulation of* F. solani *and* M. phaseoli *at 22°C.*

Initial *p*H	F. SOLANI			M. PHASEOLI		
	Dry wt. of mycelium (mg.)	Sporulation (million)	Change of *p*H after growth	Dry wt. of mycelium (mg.)	Sclerotia	Change of *p*H after growth
2.1	—	—	—	—	—	—
2.5	—	—	—	—	—	—
3.0	—	—	—	—	—	—
3.5	28	97	3.9	25	+	4.0
4.0	22	480	4.4	33	++	5.3
4.5	25	439	4.4	16	++	5.4
5.0	36	525	4.5	24	++	5.5
5.5	42	3120	5.0	29	+++	5.5
6.0	60	3840	5.4	37	+++	5.8
6.5	53	880	6.3	22	+++	6.3
7.0	30	180	6.3	20	++	6.4
7.5	38	105	6.3	22	++	6.5
8.0	24	135	6.3	20	++	6.5
8.5	24	105	6.5	20	++	6.5
9.0	24	97	6.5	18	+	6.6

Data in Table 6 show that both the organisms could grow over a wide range of *p*H 3.5 to 9.0. Optimum *p*H for both *F. solani* and *M. phaseoli* was at *p*H 6.0 above or below which there was reduction in growth and sporulation. In *F. solani* alkaline condition appeared to be less suitable for sporulation. In acidic range *M. phaseoli* tended to increase the *p*H while *F. solani* tended to reduce it.

IV. *The effect of temperature on growth and sporulation of* F. solani *and* M. phaseoli

To study the effect of temperature on growth and reproduction of the two organisms the experiment was conducted in the laboratory with *Richard's solid and liquid media* at 15°–40°C. The results of the observation are recorded in Table 7.

The data of the above table show that the optimum temperature for growth and sporulation of *F. solani* was 25°–30°C. and for growth of *M. phaseoli* 35°C., and for sclerotial formation 25°C. *F. solani* appeared to have narrower temperature range, while in *M. phaseoli* fair growth and sclerotial formation was noted even at 40°C., while it was completely inhibited in *F. solani*.

Table 7. *Effect of temperature on growth and sporulation of* F. solani *and* M. phaseoli *on Richard's medium at pH 6.0.*

Temperature	F. SOLANI			M. PHASEOLI		
	Linear growth in 8 days (mm.)	Dry wt. of mycelium (mg.)	Sporulation (million)	Linear growth in 8 days (mm.)	Dry wt. of mycelium (mg.)	Sclerotia
15°C.	18	7	—	26	23	—
20°C.	100	9	4	100	32	—
25°C.	100	13	88	100	49	+++
30°C.	100	13	93	100	72	+
35°C.	30	6	32	100	79	+
40°C.	—	—	—	100	33	+

The thermal death points of the organisms were studied in the usual way at different temperatures 30°, 40°, 45°, 50°, 55°, 60°, 70°, 75°, 80° and 90°C. for 5 minutes. It was observed that thermal death point of *F. solani* was in the neighbourhood of 60°C. whereas in *M. phaseoli* it was approximately 65°C.

V. *Effect of soil-moisture of* F. solani *and* M. phaseoli.

Study on the effect of soil moisture was carried out following Norton's (1933) method with both sterilized and non-sterilized soil adjusted to different moisture level from 10% saturation to 100% saturation. The tubes were kept in darkness and their linear growth was observed with the aid of simple microscope. The data are recorded in Table 8.

Table 8. *Effect of soil moisture on linear growth of* F. solani *and* M. phaseoli *at 25°C.*

Water holding capacity of the air dry soil = 49.7%
Moisture content of the air dry soil = 1.823%

Soil condition	Moisture content	Linear growth of *F. solani* on 10 days (mm.)	Linear growth of *M. phaseoli* on 10 days (mm.)
Non-sterile	100%	12	18
	80%	20	28
	60%	56	76
	40%	46	126
	20%	20	64
	10%	14	51
Sterile	100%	15	15
	80%	24	28
	60%	50	71
	40%	47	153
	20%	42	34
	10%	27	32

Increasing concentration of Indol-3-acetic acid upto 50 PPM the growth was decreased and sporulation was completely suppressed in case of *F. solani*. In *M. phaseoli* vegetative growth decreased progressively with increase in concentration of the same hormone but sclerotial formation was not affected. Addition of Napthalacetic acid vegetative growth of *F. solani* and *M. phaseoli* increased with increasing concentration but sporulation reduced in case of *F. solani* only. Formation of sclerotia was not affected. Bouillanne and Bouillanne (1951) observed in culture that *F. vasinfectum* was temporarily inhibited by indol-3-acetic acid at 1 gm. concentration and was definitely blocked by indol-propionic acid at the same concentration. Further this view was confirmed by Davis and Diamond (1953) that Indol-3-acetic acid reduced the growth of *F. solani* and *M. phaseoli* in culture and also suppressed the sporulation of *F. solani* completely. Manil, Bonnier and Frascelle (1949) also observed that various species of *Fusarium* tended to adjust the H-ion concentration of the culture medium to the optimum for pigment formation.

The linear growth was observed to be less in sterilized soil than in unsterilized, soil similar findings have been made by Stover (1955). Increasing soil moisture to 80% to 100% saturation or decreasing to 20% or 10% saturation growth of both *F. solani* and *M. phaseoli* decreased. This also similar to the observation made by Stover (1955) in *F. oxysporum* f. *cubense*.

Acknowledgements

The authors express their deep sense of gratitude to Indian Council of Agricultural Research for financial assistance to the Scheme of investigation of wilt disease of guava in West Bengal under which this investigation has been undertaken.

References

Bouillanne, R. and Bouillane, M. (1951). De L'influence des hormones vegetable de synthese sur is Corissance de *Fusarium vasinfectum*. *Acad. Roy. Belgique Bull. Cl. Sci.*, **37**, 557–566.

Chattopadhyay, S. B. and Sengupta, S. K. (1955). Studies on wilt on *Psidium guajava* L. in West Bengal. *Indian J. Hort.*, **12**, 1–4.

Davis, S. R. and Diamond, E. A. (1953). Inducing disease resistance with plant growth regulators. *Phytopathology*, **43**, 137–140.

Hussein, E. (1957). Fungal association. II. Cultural studies on *Rhizoctonia solani* Kuhn, *Fusarium solani* Snyder and Hansen, and other fungi and their interaction. *Ann. Bot. Lond.*, **21**, 569–585.

Manil, P. Bonnier, C. and Fraselle, J. (1949). Apropose du point isometabolique dues cultures de divers *Fusarium*. *Bull. Soc. Chem. Biol.*, **31**, 804–809.

Maclellan, W. D. (1952). Effect of temperature on the severity of *Fusarium* basal root in *Narcissus*. *Phytopathology*, **42**, 407–412.

Norton, D. C. (1953). Linear growth of *Sclerotium bataticola* through soil. *Phytopathology*, **43**, 633–636.

Pai, K. V. S. (1953). Carbon, nitrogen, and *p*H relationships to growth of soil. *Fusaria* in culture. *Proc. Indian Acad. Sci., Sec. B.*, **37**, 131–148.

Stover, R. H. (1953). The effect of soil moisture on the growth and survival of *Fusarium oxysporum* f. cubense in the laboratory. *Phytopathology*, **43**, 499–504.

———— (1953). The effect of soil moisture on *Fusarium* species. *Canadian J. Bot.*, **31**, 693–697.

Townsend, B. B. (1952). Nutritional factors influencing the production of slerotia by certain fungi. *Ann. Bot, Lond.*, **21**, 67–77.

Yokota, S. I. (1953). On several factors which affect the conidial characteristics of *Fusarium solani* (Mart.) App. et. Wr. I. On the concentration of media, temperature and duration of incubation. II. On the concentration of medium. *Bull. Tokyo Univ. For.*, **45**, 155–164; 165–180.

(Received for publication 10 September 1966)