

STUDIED ON THE BIOLOGICAL TRAITS OF PATHOGENIC FUNGI OF BROWN SPOT
(Septoria helianthi Ell & Kell.) IN SUNFLOWER

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SUMMARY

When the Septoria helianthi Ell & Kell, were cultured for 10 - 15 days in the PDA medium, there appeared black colonies with abundant pycnidia, and like peak, and with membrane. The optimum temperature for germination of pycnidiospores is between 25-28 °C. For carbon resources, maltose, sucrose, woodsugar, glucose are better; for nitrogen sources, glutamic acid, Arginine, ammonium sulphate, ammonium nitrate, sodium nitrate all could be utilized, and the organic nitrogen is better than the inorganic nitrogen. The pH is best at 4 to 6 for germination of pycnidiospores. Pycnidiospores grow better in PDA medium, simmer juice of sunflower leaves medium and V-8 fruit juice medium. The pathogenic fungus survives the winter with disease residues above ground and becomes major primary infection source. The seed of sunflower does not carry any pathogenic fungus.

INTRODUCTION

Brown spot of sunflower, Septoria helianthi Ell & Kell, is distributed in sunflower production area, specially in Jilin, Laoning, Heilongjing provinces of China, and reduce seriously yield. Infection may be severe under condition of moisture from early July to the end of August. The sunflower disease develops first on the lower leaves then to the upper leaves, and the leaves may wither and dry unnormally, and reduce yield above 50%. The disease is an important problem demanding prompt solution. For this reason, the pathogenic fungus was investigated during 1982 - 1986.

MATERIALS AND METHODS

The pathogenic fungus was a cultured fungus which were septated and purified in 1982.

Characters investigation: to inoculate the cultured fungus on media, then put them into thermotank at 25 °C, and investigated the morphogenesis of colonies, and formed situation of pycnidiospores at the third day, the fourth day, fifth day, tenth day and the fifteenth day.

Tested condition of germination for pycnidiospores: to take the cultured fungus compound the suspension liquid of pycnidiospores according to different requirements, and put them into media for forty-eight hours, then taken them test under microscope so that the data of germination of pycnidiospores are gotten.

The relation between form of pycnidiospores and temperature: Inoculated single pycnidiospores on media then cultured them in thermotank at 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, separately. Germination of single pycnidiospores, process of forming pycnidia and growing situation of hyphae were observed every day.

The relation between growth of hyphae and temperature:

To pick out fixed volume fungi mass dabbled them on media, then, cultured them in thermotank at 5 °C, 10 °C, 15 °C, 20 °C, 25 °C, and 28 °C and 30 °C, 32 °C and 35 °C, respectively. The forming situation of colonies size were investigated and measured at the fifth day, tenth day, fifteenth day separately.

The selection of media types:

To compound many kinds of media, the colonies size and growing of colonies were measured and observed under same culture condition. Tested the overwintering pathogenic fungi:

To collect the disease leaves in autumn were dried by airing in 1983, 1984, 1985, then put the dry leaves in between the mats. The mats were put into warm room, cold room, covered underneath soil of ten centimetres, upper and middle as well lower layers of haystack lived through winter. Every early mouths from January to July in the next year, the disease leaves in between mats would be fetched to culture for forty-eight hours in 2% sucrose liquid, then tested under microscope calculating germinative pycnidiospore numbers.

Examination for seed-borne pathogenic fungi:

To examine exterior and interior of seed by means of the common method.

RESULTS

1. Characters investigation:

When the pathogenic fungi were cultured at 25 °C for three days, the colonies were greyish-white and radial growth, and tawny with a little tubercle at the fourth day, and the centre of colonies becoming black with higher tubercle but the rim of colony still tawny at fifth day. Whole of colony was black, and produced abundant pycnidia, the tubercles like peak with membrane during 10 and 15 days. From bottom of culture vessel reflected light, we could see that colonies were black and mass shape without radial growth.

2. The relation between temperature and germination of pycnidiospores:

The ranges of temperature for germination of pycnidiospores were at 10 to 32 °C, the optimum of germinative temperature were at 10 to 28 °C. The pycnidiospores were able to germinate at 30 to 32 °C too, but the buds were shorter and unregular. The pycnidiospore were not able to germinate at 5 °C and 35 °C (Table 1).

Table 1. The relation between temperature and germination of pycnidiospores

Temperature (°C)	Percentage of pycnidiospores germination(%)			Average(%)
	Replications			
	1	2	3	
5	0	0	0	0
10	30.3	23.5	23.8	25.9
15	33.3	39.2	47.8	40.1
20	70.8	65.5	62.0	65.4
25	89.0	89.5	87.2	88.7
28	87.0	80.7	73.8	80.8
30	87.8	88.0	72.5	82.5
32	64.7	63.3	64.0	64.0
35	0	0	0	0

The relation between temperature and forming pycnidia as well as forming pycnidiospores:

When single pycnidiospores were cultured for forty-eight hours on PDA medium, they could germinate at 15 to 30 °C. The hyphae grew slightly at 15 to 20 °C, and thickly at 25 to 30 °C and coming to form pycnidia at the same time. After the pycnidiospores were cultured for seventy-two hours, they produced abundant pycnidia. The pycnidia were tawny with abundant new-pycnidiospores after

ninety-six hours. The newborn pycnidiaspores were able to germinate

4. The relation between growth of colonies and temperature as well as time:

The colonies could grow at 10 to 32 °C, and the optimum of temperature were 25 to 28 °C. Although colonies were bigger, produced pycnidiospores a little at 30 °C. The colony which was cultured for five days grew slowly, and grew fast from the sixth day to tenth day, but grew slowly from the eleventh day to the fifteenth day again (Table 2).

Table 2. The influence of temperature for colony growth

Temperature (°C)	cultured time (day) & Diameter of colony (mm)		
	5	10	15
5	0	0	0
10	0	4.5	4.9
15	3.1	6.6	6.8
20	3.1	8.6	9.9
25	4.7	10.5	12.4
28	5.0	10.65	11.96
30	4.7	10.7	13.1
32	0	3.0	3.1
35	0	0	0

5. Effect on utilized carbon sources for pycnidiospores:

Fifteen kinds of carbon sources had been utilized for germination of pycnidiospores, and maltoese, sucrose, wood sugar, glucose, beet sugar and lactose of them were better than other carbon sources.

Table 3. The influence of different carbon sources for germination of pycnidiospores

Carbon kind	Germinative percentage (%)	Carbon kind	Germination percentage(%)
Maltose	86.5	Levulose	48.5
Sucrose	82.0	Arabinose	45.6
Wood sugar	79.0	Raffinose	44.9
Glucose	78.2	Chrysanthumsugar	41.4
Beet sugar	71.0	L-rhamnose	30.1
Lactose	68.5	Sorbin	29.8
D-mannose	59.1	Mannose	26.8
L-sorbose	50.7		

6. The effect on nitrogen sources for germination of pycnidiospores:

Organic and inorganic nitrogen all could be utilized for germination of pycnidiospores, and glutamic acid of 1%, and arginine and aspartic acid of them were first-rate, ammonium intrate of 2% and ammonium sulphate took second place, and sodium nitrate was relatively poor (Table 4).

Table 4. The influence of difference nitrogen sources for germination of pycnidiospores

Nitrogen kind	Concentration (%)	Percentage of germination (%)	Nitrogen kind	Concentration (%)	Percentage of germination (%)
Glutamic acid	0.2	31.1	Ammonium nitrate	0.2	34.0
	0.5	37.7		0.5	37.8
	1.0	66.7		1.0	49.9
	2.0	34.6		2.0	51.9
Arginine	0.2	24.2	Ammonium sulphate	0.2	43.8
	0.5	37.7		0.5	14.3
	1.0	64.4		1.0	35.1
	2.0	34.8		2.0	47.6
Aspartic acid	0.2	22.5	sodium nitrate	0.2	21.4
	0.5	30.3		0.5	29.4
	1.0	59.5		1.0	28.6
	2.0	49.0		2.0	32.5

7. The relation between pH value and germination of pycnidiospores: Pycnidiospores were able to germination at pH 4 - 9, and the optimum pH was at pH 6 (Table 5).

Table 5. The relation between germination of pycnidiospores and pH

pH	Percentage of germination (%)	pH	Percentage of germination (%)
3	0	7	62,3
4	55	8	57.5
5	74	9	28.6
6	81	9.8	0

8. The selection of suitable medium:

The pathogenic fungi could grow on Czapek medium, Richards medium Sabouraud medium, V-8 fruit juice medium, Cornmeal food medium and PDA medium, simmer juice of sunflower leaves medium and simmer juice of spinach leaves medium. PDA medium, simmer juice of sunflower leaves medium and V-8 fruit juice medium of them were first-rate, and diameters of colonies were between 11.7 and 17.6 mm. the colonies grew slowly on other media, and diameters of colonies were between 6.1 and 7.4 mm only.

9. The result of overwintering test on pathogenic fungi: Pycnidiospores in which were covered above ground still had vital force until early July of next year. The pathogenic fungi would become major primarily infective source in sunflower production area. The pycnidiospores within leaves in which were covered upper soil for 10 cm lost vital force in early Apr. of next year. The results shown that the seed of sunflower had not carried any pathogenic fungus in exterior and interior of seed.

DISCUSSION AND CONCLUSIONS

The germination of pycnidiospores needed longer time, and the speed of germination for pycnidiospores was influenced significantly by temperature. Usually, pycnidiospores were able to germinate at 5 to 32 °C, and temperature for pycnidiospore was best at 25 to 28 °C. Germination of pycnidiospores was inhibited significantly below 5 °C and above 35 °C.

Single pycnidiospores were cultured on PDA medium for forty-eight hours, then tested them under microscope. Pycnidiospores which were cultured at 15 and 30 °C all could germinate, but hyphae grew slightly. Hyphae grew thick, at same time, beginning to form pycnidia. After single pycnidiospores were cultured for seventy-two hours, it produced abundant pycnidia. Pycnidia were twany, and produced abundant new-pycnidiospores with germinating ability after single pycnidiospores were cultured for ninety-six hours. The results shown that pycnidia and pycnidiospores might grow and form very fast under optimum conditions. Growth of colonies of pathogenic fungi and temperature were closely related. Pathogenic fungi grew slowly with smaller colony under unfavourable condition. In the growing period, colonies grew between six and ten days, and slowly between one and five days and after ten days.

The results shown that the pathogenic fungi could overwinter wherever above ground, and still possessed vital force until early July of next year. That the pathogenic fungi had overwintered were major primary infection source for brown spot of sunflower. Accordingly, surviving plants and leaves must be cleared immediately after sunflower are harvested, then sub-tillage and crops rotation to reduce the damage for sunflower production.

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