N. J. Microbiology, 2(2), 1982, pp 190 – 194

THE EFFECT OF CANDIDA TROPICALIS AND PICHIA MEMBRANAEFACIENS ON THE GERMINATION OF SEEDS OF CAPSICUM ANNUUM L.

By:

S. N. IBE, AND C. U. IKE,

Department of Biological Sciences,
University of Lagos, Lagos, Nigeria.

ABSTRACT

Inoculation of 2 x 10^6 cells of the yeasts *Candida tropicalis* and *Pichia membranaefaciens* into fruits of the large red pepper, induced soft watery rot within 4 days of incubation at room temperature, 28 ± 2°C. Germination of the seeds on wet sterile filter papers was reduced to less than 10% in one week. Abnormal germination was observed after the 4th day and dead seeds were prominent after the 6th day of infection. When seeds from 7 day old infected fruits were disinfected with 0.5% solution of sodium hypochlorite and planted on pepper agar, over 96% exhibited yeast growth, indicating that the seed infection was borne internally.

INTRODUCTION

Considerable evidence has been accumulated over the years to show that invasion of wheat squash, peas, corn and barly by storage fungi result in decrease germination of the seeds (2,3,4,6,7,10). It has also been shown that many factors including temperature, moisture content length of storage period and both the plant and fungal species affect the germinability of infected seeds (1,6,8,9,10,11). Studies by these workers (3 8) have also involved determination of the infection sites of the fungi. The site of invasion differed markedly among different seeds. It may be on the embryos or the perisperm-endosperm layer enveloping the embryos. Thus it could be surface or internal infection.

Two yeasts, *Candida tropicalis* and *Pichia membranaefaciens* isolated from spoiled Nigerian large red pepper (*Capsicum annuum* L) locally called 'tatase' were found to be agents of post harvest soft rot of the peppers. It was noted that although yeast colonies were isolated from stored grains, the effect of yeasts on seed germination has not been mentioned.

This study was therefore undertaken to determine the effect of the two yeast isolate on the germination of pepper seeds and the site of infection.

MATERIALS AND METHODS

Media. The medium used for the cultivation and viable count of yeasts was pepper agar. One hundred grams of fresh large red peppers, without the seeds, were homogenized with 500 ml of distilled water in a Waring blender. The slurry was filtered through a Whatman No. 1 filter paper using a suction pump and about 480 ml of the filtrate was obtained. Three per cent Oxoid agar No. 1 was added to the filtrate before autoclaving at 1.1 kg/cm^2 2 for 15 minutes. The pH value of the medium was 6.6

Inoculation of pepper fruits. Forty-eight hours plate cultures were used for inoculation. Stock suspensions of the cells in distilled water were prepared to give an optical density of 0.3 at 590 nm for *C. tropicalis* and at 570 nm for *p. membranaefaciens.* Aliquots of 0.05 ml of each suspension were inoculated into small sterile scalpel wounds made on ripe fruits. Control peppers received the same amount of sterile distilled water. The peppers were incubated in sterile large glass jars at room temperature, 28 ± 2°C. Viable cell count was determined by the plate count method.

Test for germination of seeds. One hundred seeds were randomly selected out of seeds harvested from the yeast infected and control fruits and planted on moist sterile filter papers in petri dishes. Each dish contained 25 seeds and was incubated at room temperature for 7 days. The filter papers were moistened daily with sterile distilled water using dropper pipets. On the 7th day, the number of germinating seeds was counted, the length of the radicle, hypocotyl and foliage leaves of the seedlings were measured.

To test for the effect of duration of infection on germination, seeds were harvested and planted on the second, fourth, sixth and seventh day after infection.

Test for infection site on the seeds. Seeds were harvested from 7 day old infected fruits and examined by the methods used in (8). Half of the seeds were surface sterilzed with 100 ml of 0.5% sodium hypochlorite solution for 10 min. rinsed five times for 5 min. in sterile distilled water and blotted dry. One hundred seeds were tested for each organism. Twenty five seeds were placed on a pepper agar plate. The plates were incubated at room temperature for 48h and examined for the characteristic yeast growth. Control seeds from non-infected fruits were similarly treated. Non-disinfected seeds were also planted on agar to determine the presence of the organisms.

RESULTS AND DISCUSSION

Effect of duration of infection on germination of seeds. When fruits of *Capsicum annuum* L. were inoculated with 2×10^6 cells of each yeast pathogen, soft rot was observed at the point of inoculation on the 4th day of incubation at room temperature, 28 ± 2°C. By the 6th day, the fruits were extensively rotted with the epicarp still intact while the pericarp was disintegrated.

The mean percentage germination of seeds planted decreased with increase in days of infection (Table 1), as observed by (4, 9) but contrary to (8) in which germination of tomato seeds was not affected by *Aspergillus* species. The highest variation in mean percentage germination was observed on the 4th day. This could be due to the fact that the soft watery rot was still confined to the area of inoculation and the number of seeds infected depended on the rate of spread of the infection. With the fruits extensively rotted by the 6th day, most of the seeds were infected and by the 7th day gerimination was reduced to less than 10%. The difference in the germinability of the seeds infected by the two yeast isolates is not significant for randomly selected samples.

The mean length of radicles, hypocotyls and foliage leaves of the germinating seeds are shown in Table 2. Seeds from 2 days infected fruits showed normal germination as control seeds with non-necrotic radicles, green hypocotyls and foliage leaves emerging. Abnormal germination of seeds was observed amongst the seedlings after the 4th day of infection. Seeds were considered to germinate abnormally if the hypocotyl or epicotyl did not emerge or if the emerging portions were necrotic as is reported in (7). Dead seeds were prominent after 6 days of infection and these were seeds from which neither the hypocotyl nor the epicotyl emerged.

Infection site on the seeds. Examination of 7 days old infected fruits revealed that a mean of 96% and 100% of the disinfected seeds exhibited the characteristic yeast growth for *C. tropicalis* and *P. membranaefaciens* respectively on pepper agar. This difference is also not significant. One hundred per cent of the non-disinfected seeds showed yeast growth for each organism whereas 0% of disinfected control seeds showed microbial growth.

The observation on the control seeds showed that the process of disinfection, rinsing and blotting dry did not introduce extraneous micro-organisms onto the seeds. The 0.5% solution of sodium hypochlorite employed should have surface sterilized the seeds (8). The infection of the seeds by both yeasts was therefore borne internally and not on the surface. The infection could be localized on the inner layers of the seed coat (7) or on the endosperm or the embryo itself (8). Further studies would be required to determine the cause of abnormal seed germination and seed death. The organisms could be producing toxins destructive to the embryo or they could be utilizing the food reserves in the endosperm faster than the embryo (7). Since *C. tropicalis* causes candidiasis in man (5), it would be interesting to find out if the mechanism of its infection is the same in plants and animals.

Finally, it is concluded that seeds from fruits of *Capsicum annuum* L. infected by the two yeasts *C. tropicalis* and *P. membranaefaciens* should not be used for planting.

Table 1.

Variation in mean percentage germination of pepper seeds with duration of infection

Duration of infection (days)	Mean % germination of seeds of	
	C. tropicalis	*P. membranaefaciens*
0	96 ± 0.5	96 ± 0.5
2	94 ± 1.0	95 ± 1.0
4	78 + 3.0	60 ± 3.0
6	16 + 1.0	10 ± 1.0
7	8 + 0.5	4 ± 0.5

Table 2.

Variation in mean length of radicles, hypocotyls and foliage leaves of germinating seeds with duration of infection.

Days of infection	Yeast Inoculated	No. of seeds planted	No. of seeds germinated	Mean length of radicles (cm)	Mean length of hypocotyls (cm)	Mean length of foliage leaves (cm)
	None	100	96	4.32	1.58	0.65
2	A	100	94	4.25	1.55	0.62
	B	100	95	4.20	1.50	0.62
	None	100	96	4.42	1.57	0.65
4	A	100	78	3.00	1.35	0.46
	B	100	60	2.80	1.22	0.42
	None	100	95	4.40	1.56	0.61
6	A	100	16	1.40	0.58	0.1
	B	100	10	1.10	0.13	0.1
	None	100	97	4.36	1.54	0.58
7	A	100	8	0.10	0.00	0.00
	B	100	4	0.10	0.00	0.00

Table 2 A = *Candida tropicalis*

B = *Pichia membranaefaciens*

LITERATURE CITED

CHRISTENSEN, C. M. 1964. Effect of moisture content and length of storage period upon germination percentage of seeds of corn, wheat, and barley free of storage fungi. Phytopathology 54:1464 – 1466.

CHRISTENSEN, C. M. 1974. Microflora and seed deterioration. *In* viability of Seeds. Edited by E.H. Roberts. pp. 57 – 93. Chapman and Hall Limited, London.

CHRISTENSEN, C. M. and KAUFMANN, H. H. 1965. Deterioration of stored grains by fungi. Annu. Rev. Phytopathol. 3:69 – 84.

CHRISTENSEN, J. J. and STAKMAN; E. C. 1935. Relation of *Fusarium* and *Helminthosporium* in barley seed to seedling blight and yield. Phytopathology 25:309 – 327.

EMMONS, C. W. BINFORD, C. H. and UTZ. J. P. 1970. Candidiasis. *In* Medical Mycology. Edited by C. W. Emmons, C. H. Binford, and J. P. Utz. pp. 167 – 182. Lea and Febiger, Philadelphia.

FIELDS, R. W., and KINGS; T. H. 1962. Influence of storage fungi on deterioration of stored pea seed. Phytopathology 52: 336 – 339.

HARMAN, G. E. and NASH. G. 1972. Deterioration of stored pea seed by *Aspergillus ruber:* evidence for involvement of a toxin. Phytopathology 62:209 – 212.

HARMAN, G. E. and PELEGER, F. L. 1974. Pathogenicity and infection sites of *Aspergillus* species in stored seeds. Phytopathology 64: 1339 – 1344.

QASEM, S. A. and CHRISTENSEN, C. M. 1958. Influence of moisture content, temperature, and time on the deterioration of stored corn by fungi. Phytopathology 48:544 – 549.

QASEM, S. A., and CHRISTENSEN, C. M. 1960. Influence of various factors on the deterioration of stored corn by fungi. Phytopathology 50: 703 – 709.

TUITE, J. E. and CHRISTENSEN, C. M. 1957. Grain storage studies XXIII. Time of invasion of wheat seed by various species of *Aspergillus* responsible for deterioration of stored grain, and source of inoculum of these fungi. Phytopathology 47:265.