Investigation on the Alternaria Rot of Akhrot (*Juglans regia* L.)

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Zusammenfassung

Als Erreger einer Fruchtfäule von Walnuss (*Juglans regia*) in Kaschmir wurde eine *Alternaria*-Art erkannt. Die Infektion erfasste 15—20% der intakten Nüsse. Werden die Schalen entfernt steigt die Infektion bis auf 40% an, wobei bis 12% Verlust an essbaren Teilen auftreten. Angaben über die Keimungstemperatur sowie über das Keimungsverhalten des Erregerpilzes sowie über wirksame Fungizide werden gemacht.

Akhrot (*Juglans regia* L.) fruit is a dry indehiscent nut with its outer shell stony hard. The senior author during his visit of Srinagar (Kashmir) in the month of September, 1972, purchased dry Akhrotes from various markets viz. Pahalgam, Kokonad, Awantipur and Dalgate etc. After breaking the shell it was found that the inner faces of the shell were having mycelial mats. About 9 to 13% of the total purchased fruits showed this association. The fungal mycelium was immediately transferred to a P. D. A. slant which revealed it to be a *Alternaria* sp. So far known to the authors no *Alternaria* sp. has been reported from this plant earlier hence this is being reported for the first time causing fruit rot of Akhrot in India. The morphological nature of the pathogen, symptoms produced, percentage infection, mode of spore germination, effect of nutrients and temperature on spore germination, thermal death point and the effect of fungicides and antibiotics have been studied in the present investigation.

The culture was maintained on solid Asthana and Hawker's medium „A" (Glucose —5.0 g, KNO_3 —3.5 g, KH_2PO_4 —1.75 g, $MgSO_4$, 7H2O —0.75 g, Agar-agar- —20 g and distilled water 1000 ml). Healthy fruits were cross inoculated in both the conditions (ie. with and without outer shell) and stored in polyethylene bags in B. O. D. incubator fixed at 30° C. At the end of incubation period the symptoms were noted and the infected portions were removed and finally weighed to determine the percentage losses incurred due to infection. Spore suspensions were incubated at different temperatures ranging between 5° to 45° C to ascertain the effects of temperature on spore germination and to find out the most suitable one. Similarly spore suspensions were prepared in tap water, 1% solution each of

glucose, sucrose, agar-agar and peptone which were left for few hours in B. O. D. incubator to find out the effect of nutrients on the spore germination. The spore suspension were filled in capillary tubes and the ends were sealed to find out the thermal death points. The sealed tubes were then tied with the bulb of thermometer end emerged in hot water kept at constant temperature for 10 minutes. Thereafter, the capillary tubes were broken with sterilized forceps in petridishes containing solid medium. Technique suggested by FORSBERG (1949) was employed to evaluate some of the fungicides. Small pieces of sterilized cotton threads were placed on Czapek-agar medium in petridishes which were then inoculated with the pathogen. The cotton threads when became covered by the fungus were subsequently rolled in a powder fungicides. The treated threads were placed then in another sets of petridishes containing solid medium and incubated at 30° C for 10 days. Efficiency of mycostatine, griseofulvin, aureofungin and ampicellin were examined at 200, 500 and 1000 ppm concentrations.

The present isolate of *Alternaria* showed thick, brown to olivaceous green mycelia which were freely septate, branched and 4.5 μ broad. 2 to many celled conidia were produced on simple (rarely branched) many celled conidiophores (Fig. 3). Conidiophores were geniculate above and measured 88—154×3—5 μ in size. The conidia were brown, muriform and measured 123—485×23—85 μ in size.

Symptoms produced. In case of early infection the inner surface of the shell showed association of the fungal mycelia which later on traversed through the clefts of the main edible part. After 7 to 9 days of incubation at 30° C the edible parts were found thickly covered with olivaceous mycelia (Fig. 1). The inner clefts of the cotyledons were also having mycelial penetration (Fig. 2). No external symptom was marked on the shell even in case of artificial inoculation.

Percentage infection. In laboratory conditions (ie. 30° C) 15 to 20% infection was marked when mycelial mats of the fungus was placed over intact fruit (with shell) whereas, 40% rotting was marked when the shell was removed before inoculation. In former case the percentage loss in weight of the edible part was negligible, while it was 12% in case of latter.

Mode of spore germination. One to several germ tubes were seen coming aout of a spore, each arising from different cells. Occasionally, a single cell produced more than one germ tube. Swelling in the germinating cells was prominent (Fig. 4).

Effect of nutrients on spore germination. The spore of the fungus germinated freely in Tap water, 1% glucose, Sucrose,

Agar Agar and peptone solutions. However, the percentage germination of spore was maximum in 1% peptone solution while, agar-agar solution favoured poorly. The relative percentage of spore germination has been given below:

Effect of temperature on spore germination. The spores of *Alternaria* sp. failed to germinate at 45° C but it germinated feebly at 5° C. Minute (projections like) germ tubes were seen at 5° C. 30° C was found to be the optimum temperature for spore germination, whereas a temperature range of 20° C to 30° C was quite favourable for spore germination.

Thermal death points. The spores of the fungus were killed at 54° C when exposed to 5 minutes, while 10 minutes exposure killed them even at 51° C.

Nutrients	% spore germination
1. Tap water	58
2. 1% Glucose Soln.	67
3. 1% Sucrose Soln.	85
4. 1% Agar-Agar Soln.	43
5. 1% Peptone Soln.	90

Effect of fungicides and antibiotics. Out of fifteen fungicides (Agrosan, Bavistin, Batrocal, Captan, Cereson, Cupravit, Dithone Z-78, Ferbam, Isothan Q-15, Onyzide, Spergon, Tillex, Thiram, Zerlate, Ziram) and four antifungal fungicides (Ampicellin, Aureofungin, Griseofulvin and Mycostatin), only Betrocal, Cupravit, Cereson, Ferbam, Tillex and Zerlate were found to be uneffective. Mycostatin and Aureofungin were found to be effective up to concentrations of 1000 ppm and 500 ppm, respectively.

Summary

A new fruit rot disease of Akhrot (*Juglans regia* L.) caused by *Alternaria* sp. was collected from Kashmir. No external symptom was marked on the outer shell. Whereas the inner cotyledons were thickly covered with mycelia. 15 to 20% infection was marked in intact fruits, while 40% infection occured when the shell was removed. In later case 12% loss was marked in the edible parts. Spores of the fungus grew well on 1% Sucrose and Agar-agar solutions. One to more germ tubes were seen emerging out of a spore. The fungus failed to germinate at 45° C, while 30° C was the optimum for its germination. The pathogen was killed at 54° C when exposed for 5 minutes, while at 51° C it was killed within 10 minutes exposure. Bavistin, Dithane

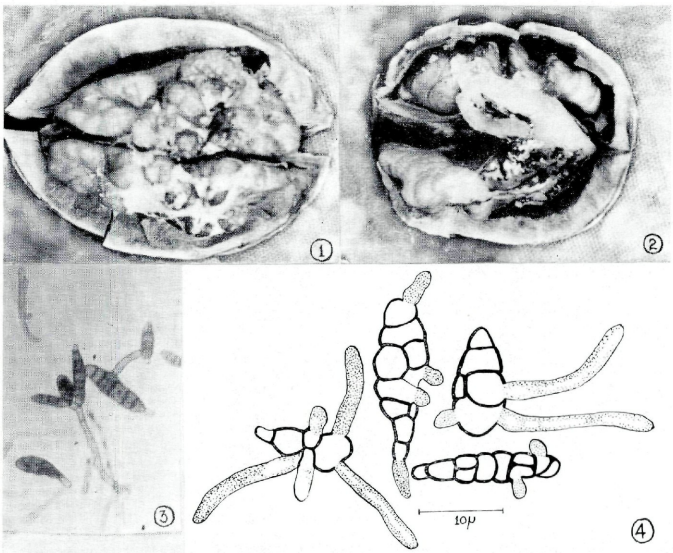

Fig. 1. Photograph of Akhrot fruit (half shell removed) showing mycetial mat over the edible part

Fig. 2. Photograph of Akhrot fruit showing mycelium penetrated through the clefts of the Cotyledon

Fig. 3. Microphotograph of *Alternaria* spores over conidiophores and germinated spores along with cauda

Fig. 4. Camera lucida diagram of the germinating spores

Z-78, Thiram and Ziram were best fungicide, while 1000 ppm of mycostatin and 500 ppm of Aureo fungin were most effective against the pathogen.

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