

STUDIES ON THE PATHOLOGICAL FRUIT DROP IN KINNOW

M. Usman Shaft, Sultan Mehmood Khan and Abdul Rehman

Department of Plant Pathology, University of Agriculture, Faisalabad

Abstract

In Pakistan, citrus is attacked by a number of fungal diseases, such as anthracnose, stem end rot, styler rot, black spot and water spot which are responsible for serious fruit losses especially in kinnow. Two fungi viz. *Alternaria citri* and *Diplodia natalensis*, are considered to be largely associated with pre harvest fruit drop. To evaluate the losses of drop in kinnow in the Bhalwal tehsil of district Sargodha a survey was conducted in seven villages of Bhalwal. The result of survey shows that the fruit drop in all the villages was significant. *Alternaria citri* and *Diplodia natalensis* were isolated and different fungicide were evaluated at different concentrations. *Alternaria citri* showed growth at all concentrations of Benlate, Daconil, Dithane M-45, Ridomil, Topsin M, and Vitavax. The most suitable fungicide for inhibition of growth of *Alternaria citri* was found Tilt which shows minimum growth at 5 and 10 ppm and completely checks the growth at 20 and 40 ppm concentration. *Diplodia natalensis* showed growth at all concentrations of Ridomil, Vitavax, Tilt, Rubigan and Daconil. Only Benlate was found to be most effective as decreased growth 84.45% and 92.23% at the concentrations of 5 and 10 ppm respectively. At the concentration of 20 and 40 ppm completely control the growth of *Diplodia natalensis*.

INTRODUCTION

Citrus belongs to the family Rutacea and consist of commercial species, as kinnow, Feutrell early, sweet orange, grapefruit and sweet lime and minor categories like tangerines, pumelos, tangelos, Kumquats, etc. essentially all the species of genus citrus are believed to have originated in the subtropical and tropical regions like southern Himalayas, South Eastern Asia, Indonesia and areas there round about, have spread to other section of the worlds (Ahmad and Ghafoor 1962).

Kinnow are very popular and earn an appreciable amount of foreign exchange. Citrus has high nutrient and refreshing value and is the best source of vitamin C, sugar, amino acids and other nutrients. In Pakistan citrus enjoys first

position among 30 other fruits. About 95% of the total production is concentrated in Punjab Province (Khan *et al.*, 1992)

It is mainly produced in districts in Sargodha, Multan and Bhawalpur of Punjab, in Hyderabad division of Sindh and Peshawar division of N.W.F.P. (Malik, 1992). In Pakistan citrus is cultivated on about 194.2 thousands hectares with annual production of 1830.3 thousand tones contributes about 8% of the worlds citrus fruits and earns a major source of foreign exchange for the country (Anonymous, 2003). The popular fruit grown in the Punjab are kinnow which contributes 70% of the entire production and is famous through out the world due to its symmetrical size and shape, fragrance, taste, soft peel, bright appetizing color.

The main objectives of research were to get survey of fruit drop and isolation, identification of associated fungi causing fruit drop, pathogenicity and in vitro chemical control.

MATERIALS AND METHODS

Survey

In order to measure the pathological fruit drop or severity of drop of kin now, a survey was conducted in the important citrus growing areas of Punjab i.e., Sargodha and Bhalwal districts. For this purpose seven villages were selected, in this way total 21 orchards were selected in seven villages. In each orchard 20 trees were selected randomly and fruit drop was recorded with the help of following scale.

$$\frac{\text{No. of fruit Drop}}{\text{Total No. of trees}} \times 100$$

For the isolation of fungi from diseased samples (fruits) Potato dextrose Agar medium was used which is prepared with the help of following ingredients.

Potato Starch	250g
Dextrose	250g
Agar Agar	20g
Distilled Water	1000 ml

While conducting the survey of kinnow orchards and recording the incidence of fruit drop, kinnow exhibiting the symptoms brought to the laboratory in polythene bags and stored at 4°C for further study. For isolation of fungi from diseased specimen Infected or part of kinnow bearing fruiting body of the fungi was cut into small pieces and then disinfected with 0.1 % Mercuric Chloride (Hgcl₂) and then there washings to reduce the effect of Hgcl₂. These pieces then plated on Potato Dextrose Agar. These Petri plates were incubated at 25°C for seven days

when fungal colonies developed on the PDA. Isolates were purified in the test tubes.

The fungi will be multiplied on PDA medium in conical flask for seven days at 25° C. Then spore suspension was prepared by adding sterile water to the culture in the ratio of 100: 40 on microscopic examination it was observed that each drop contained 70-80 spores on an average.

For in vivo inoculation, the fruits were surface sterilized with 70% ethyl alcohol and then washed with sterile water. For ripe fruits were selected on the trees and divide into lots of ten. In the first set, fruit were sprayed with spore suspension with out making an injury. In control set, spraying was made with sterile water only. In the second set fruits were injured and then sprayed with spore suspension. In the control set fruit were injured and sprayed with sterile water only. The inoculated and control fruits covered 'with polythene bags for 75% to 85% humidity\observation was recorded after 15-24 days.

In vitro trial of different fungicides. The effect of eight fungicides will be studied on isolated fungi on potato dextrose Agar in the laboratory by using dilution plate technique. Fungicides used were Daconil, Dithane M-4S, Benlate, Topsin M, Ridomil, Vitavax. Each fungicide was used at four concentrations S, 10, 20, 40 ppm. The fungicides were weighed according to their concentrations for a volume of 1000 ml conical flask; autoclaved at 121°C at a pressure of 15 lbs/sq inch for 20 minutes. Conical flasks were taken out when temperature cooled down to about 40°C. The weighed quantity of the fungicide was put in each flask mixed thoroughly and poured in sterile petri plates. These plates were then inoculated

with small piece of actively growing cultures.

RESULTS AND DISCUSSIONS

The survey was conducted in 7 village of Bhalwal, Tehsil of Sargodha, three orchards form each village. In which twenty trees randomly selected for recording of fruit drop in each tree.

In 1st village 20% trees of orchards 1, 5 % trees of orchards 2 and 10% trees of orchard 3 show zero fruit drop of kinnow, while 30% trees of orchard I , 20% trees of orchards 2 and 35 % trees of orchards 3 show one fruit drop. Two fruit drop was observed in 45 % trees of orchard I, 35% and 30% trees of orchards 2 and 3 respectively, while 5 % trees of orchards I, 2 and 3 respectively show 3 fruit drop. The maximum numbers of fruit drip in this village was four and only seen on 10% trees of orchards 2. (Table 1)

In second village 10% trees of each orchards show no fruit drop, while I fruit d was observed in 45%, 30% and 50% trees of orchards 2 and 35% trees of orchards 3 show 2 fruit drop, while 5%, 30% and 5% trees of orchards I, 2 and 3 respectively show 3 fruit drop. (Table 2)

In 3rd village no fruit drop was observed in 10%, 15% and 5% trees of orchard 1,2 and 3 respectively, while 50% trees of orchard I, 45% trees of each orchards 2 and 3 show I fruit drop respectively. 40% trees of orchards I, same numbers of trees of orchard 2 and 45% trees of orchards 3 show 2 fruit drop. Maximum numbers of fruit drop in this village was 3 and only seen on 5% trees of orchard 3. (Table 3)

In fourth village only 5% trees of orchards I. 15% trees of orchard 2 and 20% trees of orchard 3 show no fruit drop, while 40% trees from each orchard I and 2, 35% trees from orchard 3 show I fruit drop. The percentage of two fruit

drop in 45%, 40% and 30% trees of orchard I, 2 and 3 was observed respectively, while 3 fruit drop seen I 0 %, 5% and 10% trees of orchard of I, 2 and 3 respectively. (Table 4)

In fifth village only 5% trees of orchards 1, 5% trees of orchard 3 show no fruit drop of kinnow, while 45% trees of orchard I and 40% trees of orchard 2 and 35% trees of orchard 3 show I fruit drop. 2 fruit drop was observed in 30%, 45% and 45% of orchard I, 2 respectively, while 3 fruit drop seen 15 %, 15% and 10% trees of orchard of I, 2 and 3 respectively. The maximum number of fruit drop this orchard was 4 and observed on only 5% trees of orchard I and 5% trees of orchard. (Table 5)

In orchard 6th only 10% trees of orchard 2 and 15% .trees of orchard 3 show no fruit drop of kinnow, while one fruit drop was observed in 30%, 40% and 70% trees of orchard I, 2 and 3 respectively. The percentage of two fruit drop in 50%, 40% and 15% trees of orchard 1, 2 and 3 respectively, while three fruit drop showed by 15 % trees of orchard 2. In this villa e maximum number of fruit drop was 4, observed on only 5% trees of orchard (Table 6)

In 7th Village only 5% trees of orchard 1, 15% trees of orchard 2 and 10% trees of orchard 3 show zero fruit drop of kinnow. One fruit drop was observed in 60%, 50% and 55% trees of orchard 1, 2 and 3 respectively, while 35% trees of orchard I and 2 and 30% trees of orchard 3 show two fruit drop. The maximum number of fruit drop in this village was 3, which was observed only on 5% trees of orchard 3. (Table 7)

Haq (1965) estimated that citrus fruit losses were around 10-15% of the annual production i.e. 7 million mounds which value to Rs. 105 million and these go to

waste, starting from the standing tree stage to the consumption of total fruit. Naqvi and Daas (1994) conducted a survey in several districts of Maharashtra, India, and indicated that 43% and 47% of the total losses of mandarins in trucks and train's transport respectively were due to post harvest diseases. Stem end rot, *Colletotrichum gloeosporioides* and occasionally *Alternaria citri* and *Phomopsis citri* contributed to 21-26% of losses.

Isolation and identification

On potato dextrose agar at 25°C, one of the colonies of the fungus was initially showing white aerial mycelium, but later becomes light or dark gray in color. Pycnidia were subglobose to globose (300-700 Micro meter in diameter), single or grouped in stroma, and bearing spores measuring (17-43 Micro meter). Young spores were hyaline, nonseptate, and granular, and mature spores were one septate and striated. Perithecia are single or in group, globose (225-300 Micro meter). Ascospores were hyaline, one celled and elliptical to ellipsoidal (30-35. 11-14 Micro meter). The above characters agree more closely with *Diplodia natalensis*. and the organism has been identified as such.

Another colony of the fungus on potato dextrose agar showing slender aerial mycelium, which was yellowish or olivaceous hyaline. The conidia varied in size and shape and were often catenulate. At maturity, they were short clavate, oblong {(25-40)(15-22) Micro meter}, and dark olive brown. They were four to six- septate and slightly constricted at the septa and were also divided by one or more longitudinal septa. The above characters agree more closely with *Alternaria citri* and the organism has been identified as such.

Pathogenicity Test

The two isolated fungi i.e. *Alternaria citri* and *Diplodia natalensis*, were found to be highly pathogenic in causing decay of kinnow mandarian, the results were represented in table 9. It was observed that symptoms did not appear till 15th day, after which minute spots started to appear increasing in size and diameter and coalesced with each other. Finally the fruits were rotted after 22 days. *Alternaria cftri* and *Diplodia natalensis* cause 100% infection in case of injured fruits while in case of uninjured fruits it was observed 70 and 60% respectively. No infections were observed in control.

In Vitro Trial of Different Fungicides

In order to test fungi toxicity of eight fungicides against the mycelial growth of *Alternaria cftri*, food poisoned technique was used. Results revealed that in general, all the fungicide showed inhibitory effect at all concentrations. *Alternaria cftri* was found to be more sensitive to Tilt at all concentrations, which caused 84.45% decrease in mycelial growth of the fungus, followed by Rubigan and Dithane M-45 which cause 71.11 and 56.67 percent decrease in mycelil of the fungus over control at 5 ppm. It was found that inhibition in mycelial growth increased with increased in concentration of fungicide used. But at the concentration of 20 ppm and 40 ppm, out of the eight fungicides, Tilt inhibit 100% growth of the fungus, followed by Rubigan which show 95.56 and 100% results at 20 and 40 ppm respectively. Except fungicides mentioned above, remaining all fungicides performed poorly.

Diplodia natalensis. was found to be more sensitive to Benlate at all concentration, which cause 84.45%

Table 1:

Number of Fruit drop	Orchard 1	Orchard 2	Orchard 3
0	20	05	10
1	30	20	35
2	45	35	30
3	05	30	05
4	0	10	0

Table 2:

Number of Fruit drop	Orchard 1	Orchard 2	Orchard 3
0	10	10	10
1	45	30	50
2	40	50	35
3	05	30	05
4	0	0	0

Table 3:

Number of Fruit drop	Orchard 1	Orchard 2	Orchard 3
0	10	15	05
1	50	45	45
2	40	40	45
3	0	0	05
4	0	0	0

Table 4:

Number of Fruit drop	Orchard 1	Orchard 2	Orchard 3
0	05	15	20
1	40	40	35
2	45	40	30
3	10	05	10
4	0	0	05

Table 5:

Number of Fruit drop	Orchard 1	Orchard 2	Orchard 3
0	05	0	05
1	45	40	35
2	30	45	45
3	15	15	10
4	05	0	05

Table 6:

Number of Fruit drop	Orchard 1	Orchard 2	Orchard 3
0	0	10	15
1	30	40	70
2	50	40	15
3	15	10	0
4	05	0	0

decrease in mycelial growth of the fungus, followed by Topsin 45 and Oithane M-45 which cause 82.23 and 81.11 percent decrease in mycelial growth of fungus over control at 5 ppm. It was found that inhibition in mycelial growth increased with increased in concentration of the fungicide used. But at the concentration of 20 ppm and 40 ppm, out of the eight fungicides, Benlate, Topsin and Dithane M-45 inhibit 100 percent growth of the fungus at 40 ppm, followed by Dithane M-45 and Topsin which also give good results at 20 ppm. Except fungicide mentioned above, remaining all fungicide perform poorly.

Stem end rot caused by *Diplodia natalensis* and found that Benlate (500 ppm) in field reduced the intensity effectively (Brown and Albrigo, 1970). Wilfer *et al.* (1993) found that *Alternaria* stem-end rot (black rot) (*Alternaria citri*)

from these diseases can be reduced by the application of Benlate (50W 2lb/acre) pre harvest spray. It was suggested by Kumar and Grover (1964) that fungicidal spray after the pathogen had entered the fruit is not of much avail

Table 7:

Number of Fruit drop	Orchard 1	Orchard 2	Orchard 3
0	05	15	10
1	60	50	55
2	35	35	30
3	0	0	05
4	0	0	0

Table 8: Over all percentage of fruit drop in 7 villages

Number of fruit drop	Plants showing fruit drop	Percentage
0	40	9.52
1	218	42.38
2	382	39.05
3	414	7.62
4	420	1.43

can cause losses of citrus fruit. Losses

and hence the spray should be given in

the pre flowering and flowering stages. They also suggested that the application of Phaltan (5 g/ml) and Bordeaux mixture (4: 4: 50) are effective against *Alternaria cf tri*

Table 9: Effect of various fungicides on the mycelial growth of *Alternaria citri* at different concentrations

Treatments	Percentage decrease over control at 5 (ppm)	Percentage decrease over control at 10 (ppm)	Percentage decrease over control at 20 (ppm)	Percentage decrease over control at 40 (ppm)
Benlate	50	56.67	68.89	85.56
Daconil	40.45	21.11	28.89	37.78
Dithane M-45	56.67	73.33	86.67	96.67
Ridomil	5.56	8.89	13.33	18.89
Rubigan	71.11	87.78	95.56	100.00
Tilt	84.45	94.45	100.00	100.00
Topsin M	20.00	20.00	27.78	36.67
Vitavax	9.33	10.00	14.45	20.00
Control	0.00	0.00	0.00	0.00

Table 10: Effect of various fungicides on the mycelial growth of *Diplodia natalensis* at different concentrations

Treatments	Percentage decrease over control at 5 (ppm)	Percentage decrease over control at 10 (ppm)	Percentage decrease over control at 20 (ppm)	Percentage decrease over control at 40 (ppm)
Benlate	84.45	92.23	0.00	100.00
Daconil	67.78	73.33	80.00	90.00
Dithane M-45	81.11	87.78	96.67	100.00
Ridomil	48.89	54.45	62.32	71.12
Rubigan	12.22	17.78	24.45	32.22
Tilt	21.11	25.56	32.22	40.00
Topsin M	82.23	91.11	97.78	100.00
Vitavax	11.11	15.56	33.34	41.11
Control	0.00	0.00	0.00	0.00

Table 9: Pathogenicity test in orchards under natural field conditions

Fungi	Treatments	No. of Fruits inoculated	No. of fruits showing symptoms	Percentage of Infection
Alternaria cftri	Uninjured fruits	10	07	70
	Control	10	0	0
	Injured fruits	10	10	100
	Control	10	0	0
Diplodia natalensis	Uninjured fruits	10	06	60
	Control	10	0	0
	Injured fruits	10	10	100
	Control	10	0	0

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