

Pestalotia Fruit Rot on Strawberry Plants in Egypt

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Isolation from strawberry rotted fruits cvs Tamar and Yael (*Fragaria x ananassa* Duch.) which were collected from Ismailiya governorate, Egypt, in two different periods during 2006-2007 agricultural season yielded 387 fungal isolates belonging to four fungal genera. Most fungi isolated from severe infection were identified as, i.e. *Alternaria* sp., *Botrytis cinerea*, *Pestalotia longisetula*, (*Pestalotiopsis* sp.) and *Rhizopus stolonifer*. *P. longisetula* occurred at higher frequency than others. Pathogenicity test was confirmed with *P. longisetula*. It exhibited a virulent fungus under field condition causing fruit rot and severe damage especially under low temperature and higher humidity (RH) as well as rain and cool season(s). Cultivar Yael was more susceptible to infection than cv. Tamar. Pathogenicity test of *P. longisetula* on different hosts indicated that the fungus had great ability to infect apricot, peach, guava and tomato fruits causing fruit rots. Ripe and mature fruit stage was the most susceptible to infection with *P. longisetula* than the pre-mature stage. Also, infection increased as the time period of incubation under higher humidity was increased. *P. longisetula* reduce some properties of strawberry fruits. Moreover, reduced mature stage was the most reduction percent than pre-mature stage in both fresh weight as well as total soluble solids (TSS). It reduced 7.75% of fresh weight in mature stage, but decreased 0.85% of fresh weight in pre-mature stage. Also, *P. longisetula* decreased significantly the total soluble solids (TSS) content of infected strawberry rotted fruits. Decreasing total soluble solids (TSS) was increased by increasing the time of incubation.

Keywords: *Alternaria* sp., *Botrytis cinerea*, *Fragaria X ananassa*, fruit rot, *Pestalotia longisetula* and *Rhizopus stolonifer*.

Strawberry (*Fragaria x ananassa* Duch.) has been widely cultivated in Egypt. It is one of the most important vegetable crops for local consumption and exportation. It is cultivated in at least five governorates, i.e. Kalubiya, Ismailiya, Sharkiya, Behira and Menofiya. Fruit rot disease caused by fungi is one of the major problems to strawberry cultivation and production reducing their quantity and quality and causing economic losses in field, at harvest time, during marketing and exportation.

Alternaria spp., *Aspergillus* spp., *Botrytis cinerea*, *Rhizopus stolonifer*, *Rhizoctonia solani*, *Phytophthora cactorum*, *Fusarium* spp., *Penicillium* spp. and *Sclerotinia sclerotiorum* are the most fungal isolates causing strawberry fruit rots in Egypt (Khafagi, 1982; Tadrous, 1991 and Tarek, 2004).

Most species of *Pestalotia* are plant pathogens (Zhu *et al.*, 1991 and Zhang *et al.*, 2003). Studies on *Pestalotiopsis* diversity in china has revealed about 120 pathogenic and saprobic species with some new (Bhardwaj *et al.*, 1998; Chen *et al.*, 2002 & 2003; Wang *et al.*, 2002 and Zhang *et al.*, 2003). *Pestalotiopsis* is a species-rich genus occurring as pathogens, endophytes and saprophytes (Jeewon *et al.*, 2004).

Pestalotia rot of stored strawberry fruit, caused by *P. longisetula* Guba, was first noted in Israel, where it was considered insignificant as long as strawberry production was confined to the cool season, (Howard, 1973; Howard and Albregts, 1973; Lokesha and Shetty, 1993; Mass, 1998; Shitole *et al.*, 2000 and 2001 and Camili *et al.*, 2002). Embaby (2007) identified *Pestalotia* rot of strawberry fruit in some commercial plantings of cvs. Tamar and Yael in Ismailiya governorate, Egypt as first record under rainy condition.

Pestalotia rot of strawberry fruits caused by *P. longisetula* Guba, was first recorded in Israel (Howard, 1973); USA Howard and Albregts, (1973); then in India (Shitole *et al.*, 2000). *Pestalotia* sp. has been reported as the causal agent of strawberry leaf spot disease in India (Bose, 1970), and in Brazil (Camili *et al.*, 2002). In USA it causes a cortical decay of petioles and stolons of strawberry (Royse and Ries 1976) and has been isolated from diseased strawberry root in China (Zhu *et al.*, 1994).

This study was conducted to isolate and identify the pathogen(s) of strawberry fruit rot and to evaluate the susceptibility of some plant species to virulent isolate of *P. longisetula* as well as the effect of infection on some fruit properties.

Materials and Methods

1- Fruit samples:

Naturally rotted fruit samples, either of cvs. Tamar or Yael of strawberry cultivars, were collected from different localities of Ismailiya governorate during 2006-2007 seasons. Diseased fruits showing various types of rots started with watery surface lesion covered with fluffy white mould, grey- white or brown, discoloured, softening and collapsed fruits.

2- Isolation, purification and identification of the causal organisms:

Rotted fruits of strawberry cvs. Tamar and Yael were rinsed several times with sterilized water, surface disinfected by 70% ethanol, air dried and cut into small pieces. These parts were cultivated in sterilized Petri dishes contained PDA medium and incubated at 23±2°C for 3 days as mentioned by Chang *et al.* (1998). The growing fungi were isolated and purified on PDA using the hyphal tip or single spore technique then transferred on PDA slants and kept at 5°C for further studies. Isolated fungi were identified in the Plant Pathol. Dept., National Res. Centre, Egypt, according to Gilman (1957); Barnett and Hunter (1972). Also, culture characteristics, light microscope (LM) and electron microscope (EM) were used to verify the identification of *P. longisetula*.

3- Pathogenicity test:

Apparently healthy fruits cv. Yael at the commercial mature stage were surface sterilized by 70% ethanol, washed several times with sterilized water then dried and inoculated by a suspension of *Pestalotia longisetula* (10^4 CFU/ml) Chang *et al.* (1998) by using a sterile syringe. Fruits were incubated in glass moist chamber with 90-100% RH at $23\pm 2^\circ\text{C}$ according to Chang *et al.* (1998). Four replicates were used, while control treatment was injected with sterilized water only and the causal was reisolated again from diseased fruits.

4- Host range of *P. longisetula*:

Fruits of apricot (*Prunus armeniaca* L.), peach (*Prunus persica* L.) from family Rosaceae; Guava (*Pisidium guajava* L.) family Myrtaceae; tomato (*Lycopersicon esculentum* L.) family Solanaceae and bean pods (*Phaseolus vulgaris*) family Fabaceae (Legumes) were inoculated as mentioned before by a suspension of *P. longisetula* in order to study the pathogenic capability of *P. longisetula* on these fruits. Other groups were inoculated with only sterilized water as a control. Three replicates were used for each host. All tested fruits were incubated at $23\pm 2^\circ\text{C}$ and 90-100 RH (Change *et al.*, 1998) for 3-7 days.

5- Susceptibility of different mature stages to infection:

Apparently healthy mature and pre-mature strawberry fruits, cv. Yael were surface sterilized and inoculated as mentioned with *P. longisetula* then incubated at $23\pm 2^\circ\text{C}$ with 90-100% RH. Infection percent (rotted fruits) were calculated and recorded in both mature and pre-mature fruits after 2, 4 and 8 days.

6- Effect of *P. longisetula* on some fruit properties:

The effects of *P. longisetula* on fruit quantity as yield losses in fresh weight (g) were calculated after 8 days from inoculation. Also fruit quality of the strawberry fruit were studied *in vitro*. Strawberry fruits were surface sterilized, then inoculated with 1×10^4 spore suspension of *P. longisetula* (Change *et al.*, 1998) and other fruit were left with out inoculation as a control. Clear juice was used to measure total soluble solids percent (TSS%) by using a hand refractometer after 2, 4 and 8 days from inoculation in both the inoculated and un-inoculated strawberry fruits according to (Helrich, 1990).

Results

1- Isolation and identification of the causal organisms:

Rotted Strawberry fruits collected from Ismailiya governorate at two different periods (each period 3 months) with two different cultivars (Tamar and Yael) yielded 387 fungal isolates. Fungal frequency was tabulated in Table (1). Data show that, four fungal genera were associated with strawberry fruit rot. These fungi were identified as *Alternaria* spp, *Botrytis cinerea*, *Rhizopus stolonifer* and the new recorded fungus *P. longisetula* (*Pestalotiopsis*). On the other hand, *P. longisetula* was the most frequent in both periods and occurred at 32.30% frequency followed by *B. cinerea* which recorded 28.42%. Data also show that moderate frequency was recorded with *R. stolonifer* 22.48% and *Alternaria* spp. was less frequent and

Table 1. Fungal frequency of strawberry rotted fruits in two different periods during 2006/2007 season

| Fungal isolate | F.P. | | | | S.P. | | | | Total | |
|----------------------------------|-----------|-------|----------|-------|-----------|-------|----------|-------|-------|-------|
| | cv. Tamar | | cv. Yael | | cv. Tamar | | cv. Yael | | Total | |
| | T.C | % | T.C | % | T.C | % | T.C | % | T.C | % |
| 1- <i>Pestalotia longisetula</i> | 25 | 26.31 | 70 | 56.00 | 10 | 14.29 | 20 | 20.62 | 125 | 32.30 |
| 2- <i>Alternaria</i> spp. | 40 | 42.11 | 10 | 8.00 | 5 | 7.14 | 10 | 10.31 | 65 | 16.80 |
| 3- <i>Botrytis cinerea</i> | 20 | 21.05 | 20 | 16.00 | 30 | 42.86 | 40 | 41.23 | 110 | 28.42 |
| 4- <i>Rhizopus stolonifer</i> | 10 | 10.53 | 25 | 20.00 | 25 | 35.71 | 27 | 27.84 | 87 | 22.48 |
| Total | 95 | 100 | 125 | 100 | 70 | 100 | 97 | 100 | 387 | 100 |
| % | 24.55 | - | 32.30 | - | 18.09 | - | 25.06 | - | 100 | - |

F.P.= First period (Nov-Jan) , S.P.= Second period (Feb.-April), T.C.= Total No. of isolates.

occurred at 16.80%. Data also show that, the occurrence of strawberry fruit rot in the first period recorded higher fungal frequency than the second period. Also, cv. Yael was harboured higher frequency than cv. Tamar.

2- Pathogenicity test:

Pathogenicity of *P. longisetula* cause severe damage in both strawberry cultivars. Cv. Yael was more susceptible to *P. longisetula* than cv. Tamar (100% and 90%, respectively).

3- Identification of *P. longisetula* (*Pestalotiopsis*):

P. longisetula has a white aerial mycelium which is more branched and produced numerous acervuli especially in old culture Fig (1a). Fig (1b and c) shows clearly septate conidia 3-5 cells the conidia had single appendages with 2-3 branches with typical morphology, characterizing the anamorph genus *P. longisetula*. Based on symptoms Fig. (2); cultural characteristics and morphological studied by using light microscope (LM) as well as electron microscopy (SEM), pathogenicity test (Fig 3) and available literature, the pathogen was identified as *P. longisetula*. This genus was classified in Coelomycetes, Deuteromycotina, but the new classified of genus *Pestalotia* in Phylum: Ascomycota, Class: Ascomycetes, Subclass: Sordariomycetidae, Order: Xylariales, Family: Amphishneriaceae, Coelomycetous anamorph. (www.indexfungorum.org).

4- Symptoms:

Small lesions appeared on collected rotted strawberry fruits Fig. (2). The fungus develops in this lesion and covering several cms. This may lead to almost complete coverage of fruit under cool rainy conditions and/or under storage in refrigerator. In some cases, the central white area enlarged but remained dry and may be slightly sunken. In white aerial mycelia developed around the central area droplets of liquid containing spores were scattered over the fungal mycelium Fig. (3); while in others, numerous acervuli erupted through the epidermis and apart of fruit they appeared black.

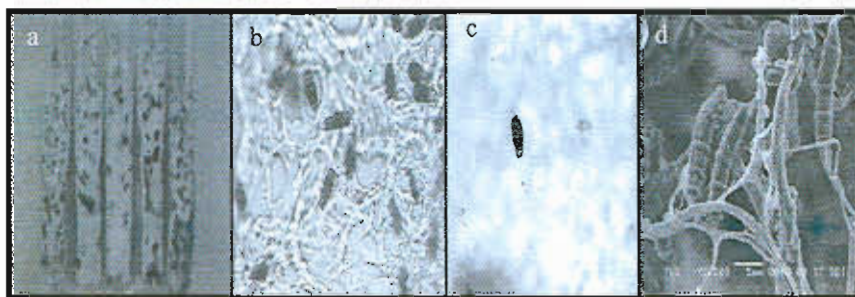


Fig. 1. Oldest culture of *Pestalotia longisetula* numerous of black acervuli (a); white aerial mycelium of which is more branched(b); conidia under LM 400X (c) and septa conidia 3-5 cells and conidia had a single with 2-3 branches by SEM 1600 X (d).

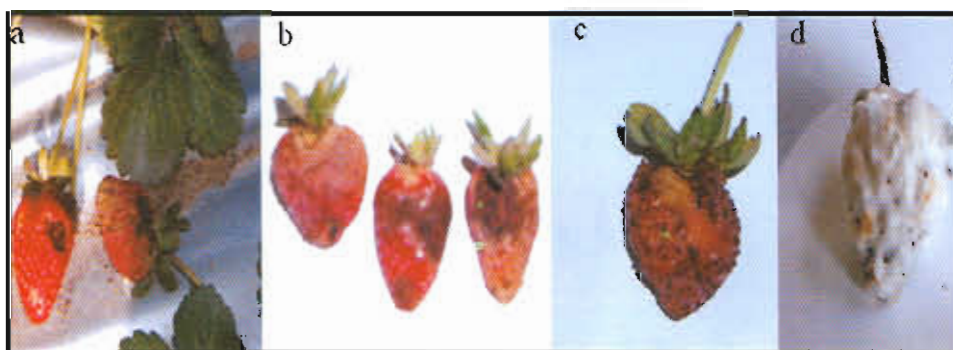


Fig. 2. Fruit rot symptoms, natural infection a, b, and c; artificial infection (d).

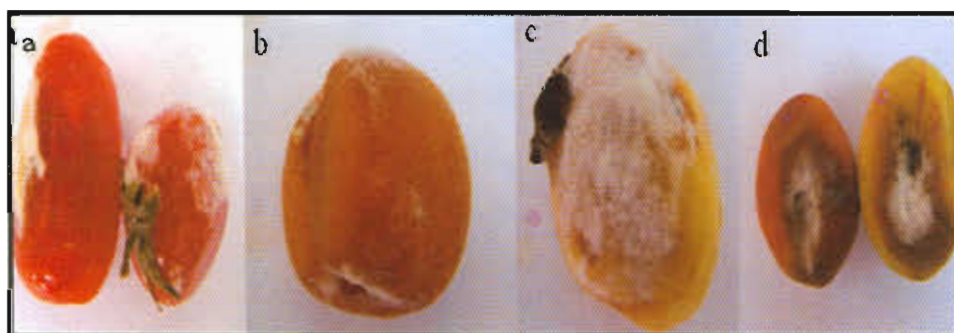


Fig. 3. Reaction of some host plants inoculated with *Pestalotia longisetula* (*Pestalotiopsis*): Tomato (a); Apricot (b); Guava (c) and Peach (d).

5- Host range:

Five plant species belonging to four families were tested for their reaction to *Pestalotia longisetula* isolated from strawberry fruit rot. Results of this reaction are recorded in Table (2) and Fig. (3). Four hosts were found to be infected and show rot symptoms. The infection zone was covered with white mycelial growth. Meanwhile, bean pods were not infected as no symptoms were obtained. The pathogen was able to attack apricot, peach, guava and tomato fruits. Percentage of infection increased by increasing the time of incubation. Disease severity appeared at different degrees according to plant species where tomato was attacked by 83.33 and 100% followed by peach by 66.67% and 100%, guava by 16.67%, 50.0% and 83.33% and apricot by 16.67%, 33.33% and 83.33%. Meanwhile, bean pods remained healthy (no symptoms).

Table 2. Host range and reaction of fruits of some plants inoculated with *P. longisetula*

| Host | | | Infection (%) after (days) | | |
|--------------|-----------------------------------|------------|----------------------------|--------|--------|
| English name | Scientific name | Family | 2 | 4 | 8 |
| 1- Apricot | <i>Prunus armeniaca L.</i> | Rosaceae | 16.67 | 33.33 | 83.33 |
| 2- Peach | <i>P. persica L.</i> | Rosaceae | 66.67 | 100.00 | 100.00 |
| 3- Guava | <i>Pisidium guava L.</i> | Myrlaceae | 16.67 | 50.00 | 83.33 |
| 4- Tomato | <i>Lycopersicon esculentum L.</i> | Solanaceae | 83.33 | 100.00 | 100.00 |
| 5- Bean | <i>Phaseolus vulgaris L.</i> | Fabaceae | 0.0 | 0.0 | 0.0 |

6- Susceptibility of fruit maturity stages:

Susceptibility of strawberry fruits at pre-mature and mature stages were tested for infection with *P. longisetula* after 2, 4 and 8 days from incubation. Percentages of rotted fruits are recorded in Table (3). Data show that, the mean percentages of infection of the pre-mature fruits were less than in the mature ones. It was zero, 27.78% and 50.0% infection percent after 2, 4 and 8 days, respectively. On the other hand, the highest infection percent was recorded in mature fruits. It recorded 44.44%, 83.33 and 100% at the three intervals, respectively. Increasing infection percent in pre-mature fruit stage from zero to 50% depends on the progress in fruit maturity during incubation for 8 days.

Table 3. Susceptibility of maturity stages for *P. longisetula* infection

| Period of incubation (days) | % Reaction / stage | |
|-----------------------------|--------------------|------------|
| | Mature | Pre-mature |
| 2 | 44.44 | 00.00 |
| 4 | 83.33 | 27.78 |
| 8 | 100.00 | 50.00 |

7- Effect of *P. longisetula* on fresh weight of strawberry fruits:

Effects of *P. longisetula* on strawberry fruit were studied in both mature and pre-mature stage. The reductions in fresh weight are shown in Table (4). Mature stage exhibited higher reduction percent than pre-mature stage. *P. longisetula* decreased 7.75% of fresh weight in mature stage of strawberry rotted fruit and 0.85% in pre-mature stage as yield loss.

Table 4. Effect of *P. longisetula* on fresh weight of strawberry rotted fruits cv. Yael after 8 days from inoculation

| Fruit stage | Fresh weight (g) | | | |
|-------------|------------------|--------|--------|------|
| | H | I | L= H-I | %D |
| Mature | 326.40 | 301.11 | 25.25 | 7.75 |
| Pre-mature | 298.77 | 296.22 | 2.55 | 0.85 |

H= Healthy

I= Infected

L = Loss

D= decreasing

8- Effect of *P. longisetula* on total soluble solids (TSS):

The effect of *P. longisetula* on total soluble solids (TSS) was studied in both mature and pre mature stage after incubation intervals (2,4 and 8 days). Data recorded in Table (5) show that, *P. longisetula* decreased significantly the total soluble solids (TSS) content in infected strawberry rotted fruits. Mature stages showed reduction percent than pre-mature stage. Decrease in total soluble solids (TSS) was more pronounced by increasing the period of incubation. The percentages of decrease were 62.56%, 67.45% and 88.61% after 2, 4 and 8 days, respectively in mature stage and 52.21%, 61.47% and 71.32% in infected pre-mature fruits in the same intervals, respectively.

Table 5. Effect of *P. longisetula* on total soluble solids (TSS) of strawberry rotted fruits

| Period/day | Mature stage | | Pre-mature stage | |
|-------------------|--------------|--------------|------------------|--------------|
| | TSS | Decrease (%) | TSS | Decrease (%) |
| 2 days | 4.37 | 62.56 | 3.25 | 52.21 |
| 4 days | 1.90 | 67.45 | 2.62 | 61.47 |
| 8 days | 1.37 | 88.61 | 1.95 | 71.32 |
| Healthy (control) | 11.67 | - | 6.80 | - |

TSS : Total soluble solids

L.S.D. 5% for TSS (a)
0.399Treatment (b)
0.564(a x b)
0.798

Discussion

Strawberries (*Fragaria X ananassa* Duch.) have been widely cultivated in Egypt. It is one of the most important vegetable crops for local consumption and exportation. Fruit rot disease caused by fungi is one of the major problems to the cultivation and production which reduced quantity and quality of strawberry productivity causing yield losses, at harvest time, during marketing, transportation and exportation.

Isolation from strawberry rotted fruits collected from Ismailiya governorate at two different dates from two different cultivars, *i.e.* cvs. Tamar and Yael yielded 387 fungal isolates belonging to four fungal genera. These fungi were identified as *Alternaria* spp., *Botrytis cinerea*, *Pestalotia (Pestalotiopsis) longisetula* and *Rhizopus stolonifer*. Similar results were obtained by Howard (1973); Howard and Albrechts (1973); Khafagi (1982); Tadrous (1991); Mass (1998) and Shitole *et al.* (2000 & 2001). Frequency of the isolated fungi was higher in the first planting than the second planting. Cultivar Yael was the most frequent in the first planting than the second planting. *P. longisetula* showed higher frequency than the others followed by *Botrytis cinerea*. *Rhizopus stolonifer* was moderately frequent, while *Alternaria* showed least frequent. Pathogenicity was confirmed on strawberry fruits.

Pestalotia species were identified based on their morphological characters (Guba, 1961 and Nag 1993). It includes approximately 220 published names (www.indexfungorum.org). Many of these were established based on slight morphological differences and host affiliation (Jeewon *et al.*, 2004).

It was virulent causing fruit rot and severe damage especially under low temp. and higher humidity as well as rainy and cool conditions. Also, pathogenicity test proved that cv. Yael was the more susceptible than cv. Tamar. Howard (1973); Howard & Albrechts (1973) and Embaby (2007) reported that, *P. longisetula* was isolated from strawberry fruit rot lesions. Rot lesions similar to those developed appeared on inoculated surface sterilized fruits.

Five plant species belonging to four families were tested for their reaction to *P. longisetula* isolated from rotted strawberry fruits. All host plants except bean were susceptible and showed rot symptoms. *P. longisetula* was able to attack apricot, peach, guava and tomato fruits. The severity of infection was increased by increasing the time of incubation period. Similar results were obtained by Hossain & Meah (1993); Sun & Cao (1993); Tsay (1993); Pandey *et al.* (1994); Badyal (1994) and Monthei (1998). Susceptibility of strawberry fruits to pre-mature and mature stage (s) to *P. longisetula* exhibited that, the main percentage of infection of pre-mature fruits was less than that of the mature ones. These confirmed the results reported by Howard (1973), Howard & Albrechts (1973), and Shitole *et al.* (2000 & 2001). *P. longisetula* reduced fresh weight of strawberry rotted fruit. Mature stage exhibited higher reduction percent than the pre-mature one. Also, *P. longisetula* decreased significantly the total soluble solids (TSS) content of infected strawberry rotted fruits. Mature stage was the most reduction percent than the pre-mature one. Rate of decreasing total soluble solids (TSS) was increased by incubation period, as reported by Dubey (1995).

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عفن الثمار البستالوشى على نباتات الفراولة في مصر

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تم عزل ٣٨٧ عزلة فطرية من عينات ثمار الفراولة (الشليك) النامية بمحافظة الإسماعيلية والمصابة بالأعفان في كل من الصنف يانيل وثمار لموسم ٢٠٠٦/٢٠٠٧. تضمنت هذه العزلات ٤ أجناس فطرية هي *Alternaria*, *Pestalotia longisetula* وكان الفطر *Botrytis, Pestalotia, Rhizopus* (*Pestalotiopsis*) الأكثر تكرارا من الأجناس الأخرى. حيث تم عزله وتعريفه والتحقق من قدرته المرضية في إحداث أعفان الثمار خصوصا تحت ظروف الرطوبة العالية والحرارة المنخفضة. كما وجد أن الصنف يانيل أكثر حساسية للإصابة بالفطر الممرض من الصنف تمار. ووجد أيضا أن جنس *Pestalotia longisetula* له مدي عوائل واسع وله القدرة علي إصابة عوائل كثيرة مختلفة مسببا أعراض مرضية في صورة عفن لثمار المشمش والخوخ والجوافة والطماطم إلا أنه لم يسبب ظهور أعفان لقرون الفاصوليا. وكانت الثمار الناضجة أكثر حساسية للإصابة من الثمار الغير ناضجة. أيضا ازدادت نسبة الإصابة بزيادة فترة التحضين بعد العدوي تحت ظروف الرطوبة العالية وبزيادة درجة نضج الثمار وتحويلها من مرحلة ما قبل النضج إلي النضج الكامل وما بعده. كما وجد أن فطر البستالوشيا *Pestalotia longisetula* يخفض من جودة ثمار الفراولة في الكم والنوع يعد الإصابة بالعفن يزداد هذا التأثير علي الثمار كاملة النضج أكثر من الثمار في مرحلة ما قبل النضج. أيضا كانت نسبة النقص في الوزن الطازج للثمار كاملة النضج ٧٥,٧% بينما كانت أقل من الواحد (٨,٨٥%) للثمار فيما قبل النضج تحت نفس الظروف ونفس فترة العدوي. لوحظ أن فطر الـ *Pestalotia sp* يخفض معنويا محتوى المواد الصلبة الذاتية الكلية في الثمار المصابة مقارنة بالمليمة. كما ازدادت نسبة الخفض بزيادة فترة العدوي من ٢ إلي ٨ أيام.