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# Effect of the Phytoplasma on Anatomical Characteristics of Sesame

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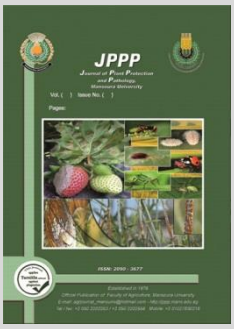


## ABSTRACT

Many economically important crops are infected with phytoplasmas, which are obligatory bacteria that do not contain a cell wall and cause great losses in crops around the world. Little information is known about the mechanisms of phytoplasma interaction with the host plants including sesame , especially on the quantity and quality of seeds' oil. Therefore, to study the effect of phytoplasma on the anatomical structure of different plant organs, , especially the capsules that contain oil-producing seeds. Samples were collected from the healthy and infected plants, which showed symptoms of phyllody, and cross sections were made in the stem, leaf and capsule. Results indicated that the thickness of the stem as well as the leaf was increased, mainly due to the increase in proportions of the vascular bundles. In the capsule, on the other hand, the pericarp shrank, the false septum dissolved in the ovary, and leaves formed instead of seeds inside the capsule. The obtained results provide a better insight on the effect of phytoplasmaon the anatomical structure of the host plant, for exploring effective ways to control the disease.

***Keywords:*** phytoplasma; anatomy; sesame, capsules

## INTRODUCTION

Sesame (*Sesamum indicum* L.) is one of the five major oilseed crops in the world and belongs to Pedaliaceae family. Sesame seeds contain high oil (29.5%–62.7%) as well as protein (13.9%–30%) contents, and is grown in more than 80 countries around the world. Latitudes between 30° S and 43° N account for over 70% of the places where sesame is grown. All of these regions are largely found in the hot, dry regions of Africa, Asia, Central America, and Latin America. Africa is a major sesame producing region (Miao *et al.*,2021). Sesame is infected with many diseases that affect the crop quantity and quality, and one of these diseases is phytoplasma, especially in Asia and Africa (Youssef *et al.*, 2018 ; Akhtar *et al.*, 2008). The disease is often transmitted by insects that feed on the vegetative parts, especially the phloem, and some parasitic plants such as dodder (El-Banna *et al.*, 2007; Ahmed *et al.*, 2014). Many symptoms appear on the affected plants, such as phyllodysis, floral virescence and proliferation of auxiliary shoots (Nakashima *et al.*,1999 ; El-Banna *et al.*,2013) which affects many vital processes such as photosynthesis, respiration, movement of stomata, and accumulation of carbohydrates (Maust *et al.*,2003; Tan *et al.*,2015 ; Ahmad *et al.*, 2019). Phytoplasma infection also reduced the trichomes of the epidermis and made the epidermal cells more sinuous (Kavathekar *et al.*,1973). In addition, infection affects the sieve elements and companion cell of the phloem, affecting phloem transport and plant growth (Christensen *et al.*,2005). The aim of the current study was to ascertain how phytoplasma affect the various anatomical features of the stem, leaf, and capsule of the sesame plant.

## MATERIALS AND METHODS

Healthy and infected samples were obtained from the sesame plant in maturity stage, cultivated in Giza Governorate. They showed phytoplasma-related symptoms like phyllody, floral virescence, the growth of auxiliary shoots, yellowing with short internodes and small leaves, and the cracking of seed capsules from germinated seeds (pls indicate how the plants were artificially inoculated if so; otherwise, indicate how is the natural phytoplasma infection is ascertained. Samples (half-centimeter) from the stem, leaf, and capsule of healthy and diseased sesame plants were killed and fixed for at least 48 h using F.A.A. (900 mL ethanol 50%, 50 mL glacial acetic acid, and 50 mL formalin) (Yeung *et al.*, 2015). Samples were dried in a series of ethanol concentrations ranging from 50% to 100%.The samples were embedded in paraffin wax using xylol as a solvent. Sections were made using a rotary microtome at a thickness of 20µm. Egg albumin was then used as an adhesive to mount the sections on slides. After the wax had been dissolved in xylol, the slides were passed through a series of ethanol solutions ranging from 100% to 50%. Canada balsam was employed to permanently mount the sections after double-staining with safranin and light green. ( Nassar, and El-Sahhar 1998; Ruzin, 1999). All photomicrographs were taken with a Zeiss microscope and a CMOS digital camera.

## RESULTS AND DISCUSSION

### Anatomical changes in the stems of phytoplasma-infected sesame plants

Infection of the plant with Phytoplasma led to an increase in the stem diameter. The rate of increase was 41% (Fig. 1-a,b and Table 1), and this was evident in the increase in all tissues of the stem including the epidermal tissue, comprising the cuticle and epidermal cells, where the increase in the thickness of the cuticle and epidermis was 30% and 53%, respectively (Fig. 1-c , d).

In addition, thickness of the cortex was increased at both the ribs and in between by 91% and 153%, respectively. This is due to not only the increase in the number of layers, but also to the increase in cortex cell dimensions. The number of cortex layers was increased by 10%, while the cortex cell diameter was increased by 40% compared to control (uninfected plants) (Fig. 1-e, f). These results are in line with those obtained by Ahmed *et al.* (2022).

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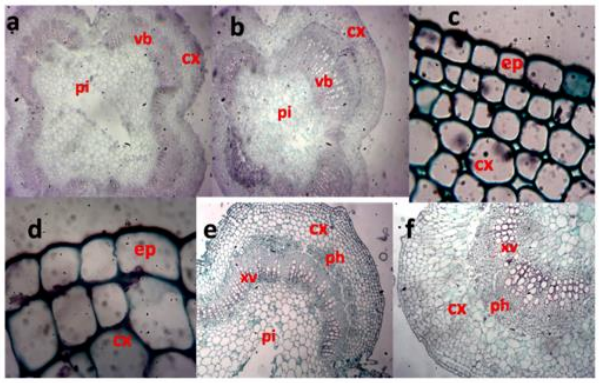


**Figure 1. Cross section of healthy (a,c,e) and phytoplasma-infected (b,d,f) sesame plants stem. cx = cortex, vb = vascular bundle, ph = phloem, xv = xylem vessels, and pi = pith. (a,b.x=100; e,f.x=150;c,d.x=400)**

The effect of the infection was also very clear on the vascular cylinder diameter, where the rate of increase was 32%, and this was accompanied with the increase in vascular tissues thickness, which reached 257%, and this increase was due to the increase in both phloem and xylem, which amounted to 193% and 245%, respectively. Xylem was thickened due to an increase in both the number of xylem vessel per arm and also to increased vessel diameter. The number of vessels increased by 55%, while the increase in the diameter of xylem vessel was increased by 89% (Fig. 1, e , f). These results are similar to those reported by Wei *et al*.(2013); Nassar *et al.* (2017); Islam and Khatoon, (2020) and Ahmed *et al*. (2022). On the other hand, pith diameter was decreased by 15%, although pith cell's diameter was increased by 33% (Fig. 1-a, b). Accordingly, the ratio of pith diameter to stem diameter was decreased by 39% in infected plants compared with healthy plants plants. On the other hand, the ratio of cortex to Pith was increased by 119% in infected plants compared with healthy plants (Fig. 1-,a, b and Table1). These results are in harmony with the findings of El-Sgai *et al.* (2020).

**Table1. Anatomical differences betwen healthy and phytoplasma-infected sesame stems.**

| | Parameters (µm) | Healthy stem | Infected stem | Change(%) |
|---|---|---|---|---|
| 1 | Cuticle thickness | 1.01-0.81-1.09 (0.97) | 1.16-1.45-1.17 (1.26) | +30 |
| 2 | epidermis thickness | 16.40-16.61-16.08 (16.36) | 27.86-21.23-26.00 (25.03) | +53 |
| 3 | Cortex thickness | 247.37-253.68-243.68 (248.24) | 465.79-494.73-460 (473.51) | +91 |
| 4 | Cortex in grove thickness | 200-199.47-172.63 (190.70) | 478.95-484.21-489.47 (484.21) | +154 |
| 5 | Cortex cell diameter | 35.98-39.52-23.44 (32.98) | 51.37-46.93-39.79 (46.03) | +40 |
| 6 | Number of cortex layers | 10-9-11 (10) | 10-11-12 (11) | +10 |
| 7 | Vascular cylinder diameter | 2060-2118- 2049 (2075.66) | 2730- 2755.01-2748.42 (2744.47) | +32 |
| 8 | Vascular tissues thickness | 228.95-243.68-276.31 (249.65) | 893.68-895.26-884.21 (891.05) | +257 |
| 9 | Phloem thickness | 74.74-64.74-63.16 (67.54) | 189.4-199.47-204.21 (197.69) | +193 |
| 10 | Xylem thickness | 213.68-176.31-178.42 (189.47) | 638.42-638.95-681.05 (652.81) | +245 |
| 11 | Xylem vessel diameter | 27.30-19.95-21.80 (23.02) | 48.94-43.91-37.51 (43.45) | +89 |
| 12 | Number of xylem vessel per arm | 5-6-7 (6) | 7-9-12 (9.3) | +55 |
| 13 | Pith diameter | 1555.79-1523.16-1632.63 (1570.53) | 1283.16-1395.79-1334.74 (1337.90) | -15 |
| 14 | Pith cell diameter | 50.42-61.21-63.91 (58.51) | 78.25-74.44-80.95 (77.88) | +33 |
| 15 | Stem diameter | 2484.74-2543.68- 2521.05 (2516.49) | 3540.12- 3538.42- 3563.33 (3547.29) | +41 |
| 16 | Pith diameter/ Stem diameter | 0.62 | 0.38 | -39 |
| 17 | Cortex thickness /Pith diameter | 0.16 | 0.35 | +119 |

**Anatomical changes in the leaves of phytoplasma-infected sesame plants**

In response to phytoplasma infection, the thickness of the affected leaf increased by 7% compared with the leaf of healthy plants (Fig. 2-a,b and Table2). Cuticle thickness on both upper and lower epidermis was increased, and the increase was 58% and 51%, respectively. On the other hand, thickness of both the adaxial and abaxial epidermis was slightly changed in response to infection. These results are similar to those reported by Atala and Moya-Urrtia (2014). Data also indicated that there was an increase in the mesophyll tissue, including both the palisade and the spongy tissue. However, this increase is mainly due to the increase in the palisade tissue, which was 44%, while the increase in the spongy tissue thickness was as low as 2%, although dimensions of its constituting sopngy cells were increased, reaching to 199% larger, compared with uninfected spongy cells. (Fig. 2-c, d).

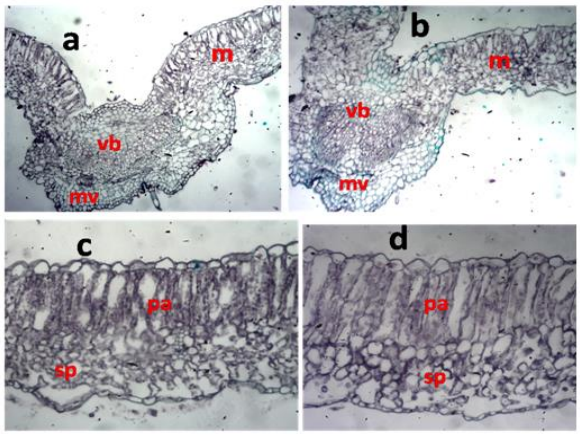


**Figure 2. vertical sections of sesame plant leaf for healthy (A,C) and phytoplasma-infected (B,D) plants.pa = palisade tissue, sp = spongy tissue, m = mesophyll, mv = midvein, and vb = vascular bundle, (a,b) . x=150 and (c,d). x= 200**

The dimensions of the midvein region revealed significant difference between infected and healthy leaves. Mid-vein length increased by 32%, whereas its width was decreased by 24%. Correspondingly, the mid-vein vascular bundle length was increased by 171 % whereas its width was decreased by 33%. These differences in the dimensions of the mid-vein vascular bundle are related to the difference in both phloem and xylem, as the phloem thickness increased by 3%, whereas xylem thickness was increased by 153%. The increase in xylem thickness is due to the increase in the number of xylem vessels, which reached 80%, despite the fact that, the diameter of these vessels was decreased by 14% (Fig. 2-a, b and Table2) .These results are consistent with the findings of Boghdady *et al.,* 2012; Mikhail *et al.,* 2012; Ahmed *et al.,*2022)who reported that phytoplasma infection affects leaf thickness and alter the proportions of mid-vein's vascular tissues.

**Table2. Anatomical differences between healthy and phytoplasma-infected sesame leaves.**

| | Parameters (µm) | Healthy leaf | Infected leaf | Change (%) |
|---|---|---|---|---|
| 1 | Cuticle on upper epidermis thickness | 0.74-0.85-0.79 (0.79) | 1.21-1.26-1.27 (1.25) | +58 |
| 2 | Cuticle on lower epidermis thickness | 0.32-0.37-0.42 (0.37) | 0.58-0.53-0.58 (0.56) | +51 |
| 3 | Upper epidermis cell thickness | 16.77-19.52-17.19 (17.83) | 18.92-16.43-19.55 (18.3) | +3 |
| 4 | Lower epidermis cell thickness | 11.80-10.05-9.36 (10.40) | 13.23-11.32-6.34 (10.29) | -1 |
| 5 | Palisade tissue thickness | 106.84-106.84-102.63 (105.43) | 153.68-148.94-154.21 (152.27) | +44 |
| 6 | Spongy tissue thickness | 128.95-136.84-117.89 (127.89) | 118.42-121.05-152.63 (130.7) | +2 |
| 7 | Spongy cell diameter | 5.55-5.82-5.13 (5.50) | 19.31-14.49-15.50 (16.43) | +199 |
| 8 | Midvein length | 587.89-672.10-524.21 (594.73) | 770-805.26-778.94 (784.73) | +32 |
| 9 | Midvein width | 1089.47-924.21-936.84 (983.50) | 751.58-731.58-745.79 (742.98) | -24 |
| 10 | Vascular bundle length | 153.68-168.42-132.63 (151.57) | 411.57-400.13-394.21 (411.39) | +171 |
| 11 | Vascular bundle width | 631.05-623.68-626.31 (627.01) | 416.84-423.15-426.84 (422.27) | -33 |
| 12 | Phloem thickness | 28.94-23.68-27.89 (26.84) | 27.89-26.80-28.01 (27.56) | +3 |
| 13 | Xylem thickness | 138.42-120.52-116.31 (125.08) | 320.52-306.31-322.63 (316.48) | +153 |
| 14 | Xylem vessels diameter | 21.16-19.84-18.83 (19.94) | 17.93-16.56-16.93 (17.14) | -14 |
| 15 | Number of xylem vessel per arm | 5-4-6 (5) | 8-9-10 (9) | +80 |
| 16 | Blade thickness | 320-271.05-312.10 (301.05) | 311.05-325.26- 326.84 (321.05) | +7 |

**Anatomical changes in the capsules of phytoplasma-infected sesame plants**

The phytoplasma infection of the sesame plant led to an evident effect on all parts of the capsule (Table.3; Fig 3-a, b, c, d, e, and f The thickness of the exocarp, mesocarp and endocarp was decreased by 30%, 52% and 77%, respectively compared with the corresponding parts in the healthy capsule. These results are consistent with those reported by Shtein *et al.* ( 2016) and Day (2000 A). At the final stages of its development, the pericarp turns into what looks like leaves with clear veins and wings (fig 3- g, h, i). the pericarp dissolves in front of the locule and begins to open and the metamorphosed leaves emerge from it (fig 3- b, e). Similar developmental alterations are reported by Akhtar *et al.* (2009) and Salehi *et al.* ( 2017). There are two main bundles in the pericarp;the first is median vascular bundle, which is found opposite to false septum, and the second which is lateral vascular bundle, which is found opposite to carpel septum and both of them were affected as a result of the infection. It was found that the median vascular bundle decreased in both length and width by 46% and 24%, respectively due to the decrease in xylem thickness, which was 43% less, although the diameter of its vessels increased by 13%. However, the median vascular bundle's phloem thickness was increased by 19% (Fig 3- j, k). Similar trend was recorded regarding the lateral vascular bundle where it was decreased in length by 20% and in width by 27%, its xylem thickness decreased by 26% despite the increase in the diameter of the vessels by 34%, and phloem thickness was increased by 24%(fig 3- l, m). The thickness of the false septum between the locules decreased by 27%(Fig. 3 k). At later developmental stages, the false septum dissolved and faded, affecting the size of the locules where locule length was increased by 16%, whereas its width was increased by 64%. Eventually, the false septum completely dissolve, uniting the openings of the locules to each other (fig.3-d,e,f,k). This corresponds to the developmental pathway described by Day (2000 a) and Day (2000 b). The infection also affected the size of the placenta, where its length increased by 93%, while its width increased by 140%. The placental vasculature comprise a number of bundles scattered throughout the placenta (Fig. 3n, o). The increase in the placental dimensions was accompanied with 29% increase in of funiculus diameter (Fig. 3,p). The irregular division of the cells increased at the end of the funiculus in the area of embryo formation, and soon the formation of leaves begins, where a leaf is formed at the beginning (Fig. 3,q), followed by another leaf below and attached to the former one (Fig. 3, r) Finally, a group of leaves linked together forms (Fig. 3, s). Similar phytoplasma infection-induced anatomical modifications were reported (Mehalingam, 2012; Ikten, *et al.,* 2014; Salehi *et al*., 2017).

From the quantitative point of view, phytoplasma infection increased capsule dimensions where radius to epicarp via carpel septum was increased by 24%, radius to epicarp via false septum was increased by 14%, and maximum capsule radius was increased by 6% (Table. 3).

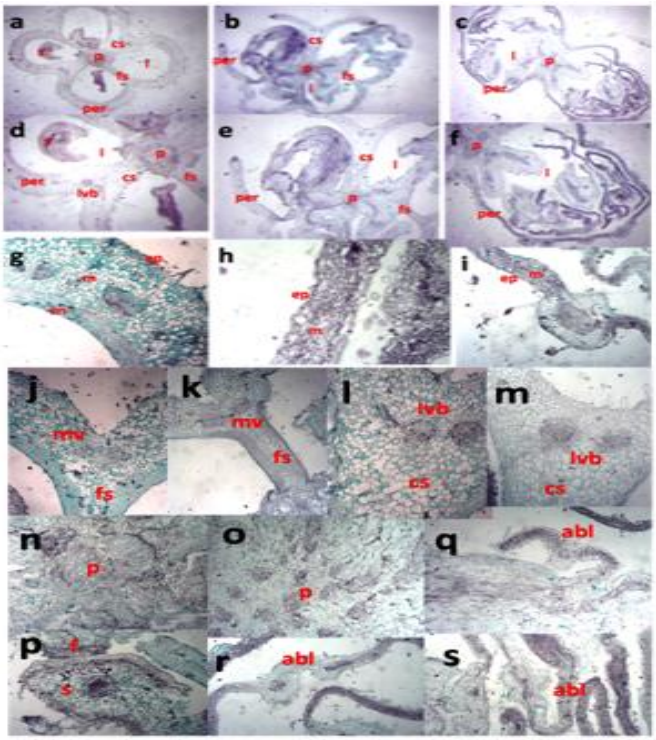


**Figure 3. Cross section in sesame plant capsules from healthy (a,d,g,j,l,n,p) and phytoplasma-infected (b,c,e,f,h,I,k,m,o,q,r,s) plants. per= pericarp, ep= Epicarp, m= Mesocarp, en= endocarp, p= placenta, l= locule, cs= carpel septum, fs= false septum, s= seed, mv= median vascular, lvb= lateral vascular bundle, abl= abnormal leaf . (a,b.c=20x; d,e.f=50x;h,i,j,k,l,m,n,o,q,p,r,s=100x; g =150x).**

**Table3. Anatomical differences between healthy and phytoplasma-infected sesame capsules.**

| Parameters (µm) | Healthy capsules | Infected capsules | Change(%) |
|---|---|---|---|
| Epicarp thickness | 26.40-19.47-20.21 (22.03) | 11.43-16.24-15.44 (15.44) | -30 |
| Mesocarp thickness | 537.89-491.57-509.47 (512.97) | 214.73-290-236.84 (247.19) | -52 |
| endocarp thickness | 168.94-161.57-161.05 (163.85) | 43.16-36.84-34.73 (38.24) | -77 |
| median vascular bundle length | 172.76-172.34-174.46 (173.18) | 84.04-99.78- 97.87 (93.89) | -46 |
| median vascular bundle width | 222.34-231.70-223.40 (225.81) | 182.34-163.83-167.02 (171.06) | -24 |
| Xylem of median vascular bundle thickness | 104.25-103.19-97.02 (101.48) | 54.68- 64.04- 54.04 (57.58) | -43 |
| Xylem vessels of median vascular bundle diameter | 20.95-16.08-18.51 (18.51) | 22.22- 18.30- 22.01 (20.84) | +13 |
| Phloem in median vascular bundle thickness | 29.78-30.85-25.31 (28.64) | 37.02-33.40- 31.48 (33.96) | +19 |
| lateral vascular bundle length | 200.85-212.04-198.81 (203.90) | 172.12-154.04-161.70 (162.62) | -20 |
| lateral vascular bundle width | 315.74- 318.64- 313.31 (315.89) | 230.21-229.57-229.78 (229.85) | -27 |
| Xylem of lateral vascular bundle thickness | 119.36-146.38-139.57 (135.10) | 105.74-99.15- 93.19 (99.36) | -26 |
| Xylem vessels of lateral vascular bundle diameter | 11.05-11.37-12.91 (11.77) | 14.81-14.39- 17.98 (15.72) | +34 |
| Phloem in lateral vascular bundle thickness | 45.10-40.85-34.04 (39.99) | 52.34-62.13- 34.25 (49.57) | +24 |
| false septum thickness | 357.36-348.94-325.79 (344.03) | 255.78-250.52-242.63 (249.64) | -27 |
| locule length | 2479.47-2487.01- 2468.45 (2478.31) | 2872.10-2880.23-2871.03 (2874.45) | +16 |
| locule width | 2177.89-2130.33-2190.72 (2166.31) | 3543.68-3530.41-3570.25 (3548.11) | +64 |
| placenta length | 885.79-895.79-884.74 (888.77) | 1708.95-1713.54-1730.11 (1717.53) | + 93 |
| placenta width | 933.68-928.94-959.47 (940.69) | 2268.42-2250.15-2260.68 (2259.75) | +140 |
| funiculus diameter | 585.26-527.36-515.26 (542.63) | 708.42-694.73-702.63 (701.92) | +29 |
| RC :radius to epicarp via carpel septum | 2142.63-2138.16-2157.12 (2145.97) | 2680-2673.55-2649.64 (2667.73) | +24 |
| RF, radius to epicarp via false septum | 2852.63-2863.14-2934.01 (2883.26) | 3293.15-3287.22- 3307.18 (3295.85) | +14 |
| RM, maximum capsule radius | 4028.94-4030.64-4018.17 (4025.91) | 4271.57-4291.26-4270.30 (4277.71) | +6 |
| VBE, distance from median vascular bundle to epicarp external wall | 137.89-118.94-120.52 (125.78) | 131.05- 125.67-122.62 (126.44) | +1 |

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## تأثير الفيتوبلازما على التركيب التشريحي للسمسم


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### الملخص


تصاب العديد من المحاصيل المهمة اقتصاديًا بالفيتوبلازما ، وهي بكتيريا اجبارية التطفل، لا تحتوي على جدار خلوي وتسبب خسائر كبيرة في المحاصيل حول العالم. لا يُعرف سوى القليل من المعلومات حول آليات تفاعل الفيتوبلازما مع النبات العائل وتأثيره على محصول السمسم ، وبالتالي على كمية الزيت وصفاته. لذلك كان من المهم معرفة تأثير الفيتوبلازما على الأجزاء التشريحية المختلفة لنبات السمسم ، وخاصة الكبسولات التي تحتوي على البذور المنتجة للزيت. تم جمع عينات من النباتات السليمة والمصابة والتي ظهرت عليها أعراض الاصابة ، كما تم عمل دراسة تشريحية لأجزاء النبات المختلفة من الساق والورقة والكبسولة. أوضحت النتائج زيادة فى سمك الساق والأوراق ، ويرجع ذلك في الغالب إلى الزيادة في بعض أجزائها مثل الحزم الوعائية. وفي الكبسولة تقلص الغلاف الثمرى ، وتحلل الحاجز الكاذب في المبيض ، و تشكلت الأوراق بدلا من البذور داخل الكبسولة. وستوفر النتائج التي تم الحصول عليها فهمًا أفضل لتأثير المرض على التركيب التشريحي للنبات المصاب ، مما يساعد على تحديد طريقة فعالة لمواجهة المرض.