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Simulation of the spreading trend of Camellia oleifera Anthracnose in Guangdong Province

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Abstract: Camellia oleifera is a unique woody oil plant in China, which has important economic value. With the continuous development of Camellia oleifera industry, the cultivated area of Camellia oleifera is expanding year by year, and the occurrence of Camellia oleifera diseases is becoming more and more serious. A variety of diseases of Camellia oleifera reported were summarized, including 51 pathogens, such as fungi, bacteria, parasitic plants, nematodes, lichens and mosses. Among them, fungal diseases are the most harmful diseases of Camellia oleifera, with 30 species, followed by parasitic plants. Different diseases types of Camellia oleifera forests were investigated month by month by using standard plot survey method in three cities, the main producing areas of Guangdong Province (Shaoguan, Heyuan, Meizhou). According to the investigation, there were seven main diseases that harm Camellia oleifera in Guangdong Province, such as anthracnose, soft rot, tea cake disease, coal soiled disease, rotting foot disease, half mad and root rot of Camellia oleifera, etc. The occurrence and damage degree of these diseases were seasonal obviously. Through the comparative investigation of different types of Camellia oleifera forests, such as nursery, new afforestation, young forest, mature forest, scion orchard, old forest and low-grade forest, the disease occurrence characteristics of different types of Camellia oleifera forests were summarized, which provided scientific basis for timely and accurate control of Camellia oleifera diseases. In addition, the pathogens of anthracnose, gray spot and withered shoot of Camellia oleifera were different in different areas, and the occurrence and damage degree of the same disease in different areas were also different. For the prevention and control of Camellia oleifera disease, it is necessary to master the occurrence and development law of the disease, reasonably select the pesticide and application period, and take the comprehensive control strategy of prevention as the main and combined with various prevention and control measures.

1. Introduction

Camellia oleifera is a unique and important woody edible oil tree species in China, mainly distributed in 14 provinces and autonomous regions which is south of Qinling mountain-Huaihe

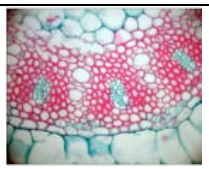
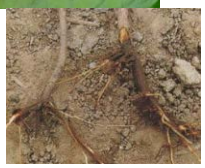
River. Colletotrichum gloeosporioides is one of the main diseases of Camellia oleifera. In the previous study, the method of morphological characteristics and multi gene sequencing identification found that, the pathogen of Anthracnose in Camellia oleifera forest mainly belonged to the member of Colletotrichum gloeosporioides complex group, which were fruit anthrax, Siamese anthrax, Colletotrichum gloeosporioides, Camellia anthracnose, Colletotrichum harreri and Colletotrichum kaster. Among them, the isolation rate and distribution range of Colletotrichum gloeosporioides were most widely [1]. Anthracnose is the main disease of Camellia oleifera, which occurs widely in the cultivation area of Camellia oleifera in Guangdong Province. At present, the species of anthracnose pathogens in Camellia oleifera nursery are not clear, and it is not clear whether there are differences in the resistance of different kinds of Colletotrichum to carbendazim, acetochlor and tebuconazole [2]. In our study, AP mat gene sequence was used to identify the species of Colletotrichum in Camellia oleifera nursery in China, and to further reveal the current situation of resistance of Colletotrichum gloeosporioides in Camellia oleifera nursery, and also reveal that is there a correlation between pathogen species and fungicide sensitivity, so as to provide basis for the prevention and control of Colletotrichum gloeosporioides.

2. Symptom characteristics of Camellia oleifera Anthracnose in Guangdong Province

It is reported that the annual yield of Camellia oleifera seeds is reduced by 10% ~ 30% and 40% ~ 50% in the seriously affected areas. In the typical stands, the diseased buds accounted for 26% ~ 45% of the total number of fallen buds. Although the late diseased fruits can be harvested, the oil content of seeds is only half that of healthy seeds, or even lower. Fruit, shoot and leaf of Camellia oleifera can be infected [3].The typical spot on the fruit is dark brown or brown round spot. In the early stage, brown spots appear on the peel, and in the later stage, the small black spots are the conidial disc of the pathogen. After rain or dew infiltration, sticky pink conidia pile is produced. When the disease is serious, several disease spots will synthesize dark brown big spots, and the diseased fruit will fall off or crack. The young leaf disease spot is mostly in the leaf margin or leaf tip, showing semicircular or irregular shape, dark brown, with water ripple wheel pattern, and the edge is purplish red [4]. The old leaf spot is sunken, brown, often with irregular and sparse whorl pattern. The edge of the lesion is purplish red brown, and the center is gray white, and there are small black spots in the center. Flower bud and leaf bud were damaged, and the disease spot mostly occurred at the base of scale, which was irregular, black and black brown. At the later stage, it was grayish white with yellowish brown inside, with black spots on it. The conidia pile was often on the inner side of the scale. At the early stage of spring shoots, most of them occurred at the base, with brown spindle or tongue shaped spots. In the later stage, oval spots appeared in the middle and upper parts of spring shoots [5]. When the diseased shoots extended to more than 2 / 3 of the stem circumference of the branches, the spring shoots showed water loss and wilt, and finally died. There are many kinds of diseases of Camellia oleifera, including fungal diseases, bacterial diseases, parasitic plants and plant pathogenic nematodes. Thiophanate methyl or carbendazim (benzimidazoles) and tebuconazole were used to control Anthracnose in Camellia oleifera nursery.Table 1 summarizes the kinds of diseases of Guangdong Province Camellia oleifera reported in the literature**.**

Table 1 Source categories of Camellia oleifera Anthracnose in Guangdong Province

type	Diseases and pathogens	Hazardous parts
Bacterial diseases	Bacterial wilt of Camellia oleifera	vascular bundle

Parasitic plants	Scurrula parasitica	 leaf
-	Camphor parasitism	  root
Plant pathogenic nematodes	Chestnut parasitism	 Fruit
Non infectious diseases	Oncosis of Camellia oleifera	Branch and trunk 

Based on the research, the infection sequence of Anthracnose of Camellia oleifera was tender shoot, tender leaf, fruit, flower bud, leaf bud and flower in early winter. In spring, the first part of the disease was the new shoots, and the next year's mycelium was the main source of new leaf infection. The peak period of fruit disease occurred at the time of fruit ripening, which was later than that of young leaves and new shoots [6]. The peak period of leaf bud and flower bud is at the initial stage of differentiation. Camellia anthracnose is a fungal disease. It overwinters with mycelium and conidia, and conidia propagate and propagate by wind, rain and insects. Therefore, site environment and variety types have a close impact on the occurrence of the disease. The Anthracnose of Camellia oleifera occurred seriously when the temperature was above 20 ℃ and the rainfall was large. Higher management level, reasonable stand structure and application of potassium fertilizer can significantly reduce the disease. There is a certain correlation between the Anthracnose and the rainfall in summer and autumn. The disease is serious because of long rain days and heavy rainfall. In addition, spring temperature and disease resistance of Camellia oleifera were also related to the occurrence of anthracnose.

The disease resistance of Camellia oleifera was mainly affected by genetic factors, but also affected by the nutritional status of the host to a certain extent. In addition to partial application of nitrogen fertilizer, the number of fruit bearing of Camellia oleifera was also related to the degree of disease. There are many fruits in Danian Camellia oleifera, and the disease is also serious [7].If there is no corresponding disease prevention measures for the well reclaimed and tending stands, the yield of fruit will be increased and the disease will be aggravated at the same time. It is connected with the vessel of vascular bundle of Camellia oleifera to absorb water and inorganic salt of host plant. Because of the downward extension of the root, the parasitic mulberry often forms a chicken leg shaped tumor.In the season of frequent and abundant rainfall, Camellia oleifera is also vulnerable to the damage of Phytophthora leaf spot. In the old Camellia oleifera forest, it is easy to be harmed by lichen moss. The occurrence of lichen moss and algae spot disease is mainly related

to climate and improper management. At present, there are few studies on lichen moss, and this paper does not focus on the description. Fungal diseases are the most diverse and most serious diseases [8]. There are two kinds of diseases caused by exobasidiomycetes and Discomycetes, and one disease is mainly caused by other fungi. According to the degree of damage, fungal diseases can be divided into three categories: anthracnose and soft rot of Camellia oleifera occur in many places, causing great economic losses; Anthracnose, root rot, leaf swelling, red leaf spot and root knot nematode of Camellia oleifera occur sporadically or locally, and the harm is relatively light; the other diseases only occur in individual places. According to the epidemic characteristics, it can also be divided into high temperature and high humidity type (Camellia anthracnose, etc.), suitable temperature and high humidity type (Camellia oleifera soft rot) and low temperature and high humidity type (Camellia oleifera leaf swelling disease, etc.). However, no matter what type of disease can be epidemic, it is closely related to precipitation or fog and dew conditions in different years, which fully shows that humidity is an important ecological condition for the epidemic of Camellia oleifera [9].In this paper, the fungal diseases of Camellia oleifera can be simply divided into leaf disease, branch bud disease, stem and root disease. Although there are many fungal diseases, the most serious ones are anthracnose, soft rot, bud disease and half witches' broom of Camellia oleifera.

3. Analysis on the spreading trend of Camellia oleifera Anthracnose in Guangdong Province

Based on the analysis of multi gene sequences of Colletotrichum gloeosporioides in Camellia oleifera and other plants, the genetic variation law of Colletotrichum gloeosporioides in different plants was revealed. Whether there was gene flow, genetic differentiation and host specificity of Colletotrichum gloeosporioides in Camellia oleifera and other host plants was clarified from the molecular level [10] .Based on this, the diffusion model of Anthracnose in Camellia oleifera in Guangdong Province was constructed as follows.

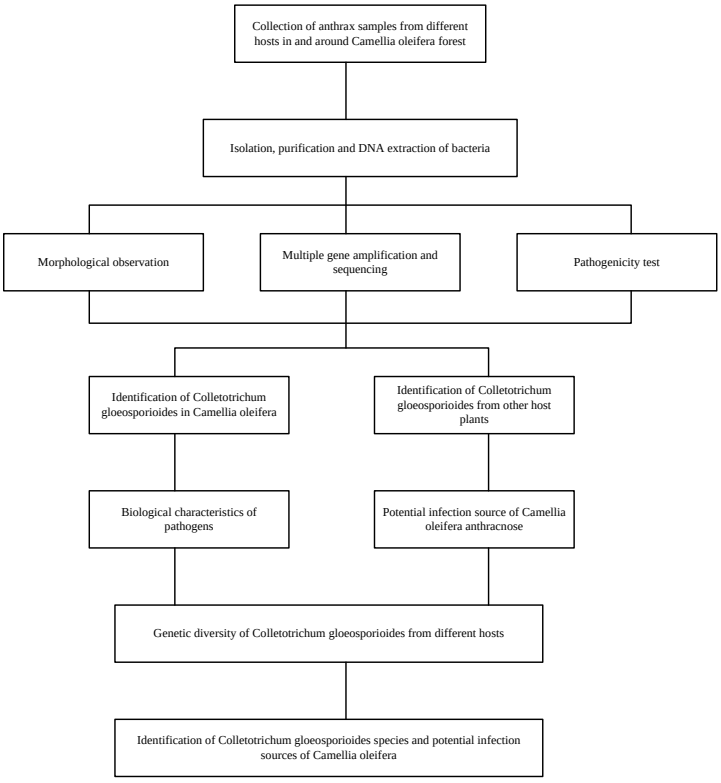


Fig. 1 Diffusion model of Camellia oleifera Anthracnose in Guangdong Province

The pathogen overwinters mainly in the infected parts of Camellia oleifera by hyphae or conidia. The overwintering pathogen on the tree body is the main source of primary infection in the next year. The conidia of Colletotrichum gloeosporioides are buried in the conidia gel. They must be dispersed by rain water and dew, and then spread by the rain water splashing and wind blowing in the rain. The pathogen can penetrate the outer layer of tea fruit and infect seeds, and the seeds of diseased fruits can carry bacteria inside and outside [11]. Anthracnose of Camellia oleifera has latent infection characteristics. It can be infected before the host organ matures or when it is young. It can be embedded in the surface wax layer of the host with appressorium, or it can infect the silk under the cuticle or in the epidermal cells [12]. The pathogen can infect the young fruit before winter and hibernate in the host tissue. The damage order of each organ of Camellia oleifera in a year is first the tender shoot, the tender leaf, then the fruit, then the flower bud, leaf bud, and finally the flower in early winter.The new shoots were the earliest part of the disease in spring. In Anhui and Zhejiang Province, the disease spots of spring shoots first appeared in late April, that is, shortly after the leaf exhibition of Camellia oleifera in spring. At the end of May, the development of the disease gradually stopped [13]. Some young leaves began to show symptoms almost at the same time of spring shoot onset, that is, shortly after leaf spreading. The latent hyphae in leaf buds were the main primary infection source of new leaf disease in the next year[14]. The onset period of fruit was later than that of new shoots and young leaves, and the peak period of disease usually occurred in the ripening period of fruit, that is, August and September. Leaf bud and flower bud could be infected by the pathogen in the first and middle of June. The peak period of the disease occurred in August. Under normal circumstances, the incidence rate of low mountains, foot, forest edge and forest is high. The incidence rate of alpine, mountain, forest and young forest is low, and the species of oil tea camellia are not suitable. The excessive nitrogen fertilizer application during the onset stage often aggravates the disease. Different varieties of Camellia oleifera have different disease resistance [15]. Camellia oleifera has strong disease resistance, while common Camellia oleifera is susceptible. Purple red fruit has stronger disease resistance than green peel fruit.

4. Simulation experiment on the spreading trend of Camellia Anthracnose in Guangdong Province

The infected leaves were collected during the peak period and brought back to the laboratory for standby. Anthrax was isolated from diseased leaves of Camellia oleifera by conventional tissue isolation method. At the junction of the diseased leaf and the healthy leaf, a small piece (3 mm × 3 mm) of diseased tissue was cut from the diseased leaf. After cleaning, disinfect with 70% ethanol for 45s, then sterilize with 0.1% mercuric chloride solution for 45s, and then rinse with sterile water for 1min. Finally, the leaves were pasted on the PPDA plate and cultured under constant temperature and continuous light at 25 ℃. When the bacteria grow backward, the tip of mycelium is picked from the edge by pricking method and moved to the fresh PPDA plate [16]. Preparation of spore suspension: after 5 days of culture under the same conditions, the colony was scratched with a sterile inoculation needle on the sterile operating table to stimulate the production of conidia. After 10 days, the pink conidia were picked with sterile inoculation needle and diluted to 103 conidia / ml with sterile water. Preparation of mycelia: the diluted conidia suspension was coated on the PPDA plate and cultured under constant temperature and continuous light at 25 ℃. When the growth of bacteria is backward, the single conidia are selected for purification. The purified strain was stored on PPDA slope and stored in refrigerator at 4 ℃. The tested strains were transferred to fresh PPDA plate and cultured under constant temperature and continuous light at 25 ℃. The colony morphological characteristics of the tested strains were recorded daily on the PPDA plate. The same scratch method was used to stimulate the production of conidia to prepare suspension. The slides

were observed under light microscope. The size of conidia was measured and the morphological characteristics of conidia were recorded.

The pathogen of Colletotrichum gloeosporioides isolated from different sampling sites were inoculated into healthy oil tea leaves respectively. Dark brown spots appeared at the stab wounds of infected leaves two days later, and then gradually turned into black spots; The disease of leaves inoculated without injury was slow. Brown spots appeared in leaves after 3 days, and then gradually developed into dark brown or black spots. No matter whether inoculated with or without injury, the leaves infected with sterile water did not. The disease symptoms of all leaves were similar. The pathogen could infect the leaves more quickly because of damaging the tissue of oil tea leaves when inoculated with wound, and the area of lesion with wound inoculation was larger than that without injury inoculation [17]. In the early stage of the disease, the leaves were dark brown, then gradually deepened, and the disease spots gradually expanded. When the humidity was high enough, the disease spots on the leaves would appear orange red or light red meristematic sachets. The results showed that the pathogen isolated from Camellia oleifera was the pathogen of Camellia anthracnose.

In Guangdong Province, Shaoguan, Qingyuan (Xianglin series, YUELIAN series, Tiecheng No.1 common Camellia oleifera), Heyuan (Ganzhou Camellia oleifera series, Changlin series, Ganwu series, Yalin series ordinary Camellia oleifera), Meizhou (Cenxi soft branch series ordinary Camellia oleifera), Zhaoqing (Guangning Camellia oleifera), Yunfu (Guangning Camellia oleifera) were investigated regularly in Camellia oleifera nursery and pure forest from April to may, Leaves of different Camellia oleifera varieties [18]. Diagonal method was used for sampling. Six spots were extracted from each variety. 20 (seedlings) or 10 (growing plants) were investigated at each site. The number of sick leaves per plant was calculated and the incidence rate was calculated. The disease resistance grade was classified according to the indexes shown in Table 2

$$A = (N / M) \times 100\% \quad (1)$$

The incidence rate was A, the number of the investigated leaves was N, and the total number of leaves was M. On the PPDA plate, the colonies were white at first, and the hyphae were sparse and dense [19]. With the extension of culture time, the colony was gray white and the back of colony was brown. After 5 days of culture, the front surface of the colony was round, light black, villous, and the edge was white. The strain rarely produced spores when cultured on PPDA plate.However, when the colony is damaged, scattered orange red conidia are easily formed around the wound. The conidia are short rod-shaped, single celled, colorless, and mostly $(13 \pm 2)\ \mu m \times (3 \pm 0.5)\ \mu m$.

Further, the grades of resistance to Anthracnose of Camellia oleifera were classified as follows:

Table 2 Grading standard for Anthracnose Resistance of Camellia oleifera

Incidence rate /%	Types of disease resistance	Representative symbol
≤ 5	Highly resistant varieties	R
6-15	Moderately resistant varieties	MR
≥ 16	Susceptible varieties	S

Based on the above table, the inhibition of Fungicides on the mycelial growth of pathogenic bacteria can be determined by mycelial growth rate method during the treatment of Camellia oleifera against Colletotrichum gloeosporioides. Firstly, sterile water was used to prepare a certain concentration of mother liquor, and diluted into a series of concentration gradients. According to the ten fold dilution method, 1ml of the drug solution was taken by sterile pipette and added into 9ml of melted PDA medium to make the medium plate with virus. A 6 mm diameter puncher was used to take the bacterial cake at the edge of the colony cultured for 5 days. The cake was inoculated in the center of the drug containing medium plate, and one bacterial cake was attached to each plate. The PDA medium without medicament was used as control, and each treatment was repeated three times. Culture in incubator at 25 ° C_ after 5 days, the colony diameter was measured by cross method and

the relative inhibition rate (%) was calculated. The logarithm of the concentration of the fungicide was taken as X, and the probability value of the relative inhibition rate of mycelium growth was y [20]. The regression equation of virulence was obtained by DPS software (v3.01)

$$F=\frac{(r_1-r_2)(r_3-r_2)}{r_1-r_3} \quad (2)$$

The relative inhibition rate (%) was f, the diameter of control colony was R1, the diameter of bacterial cake was R2, and the diameter of treated colony was R3. According to the colony morphology, growth rate, conidia and appressorium morphology of the strains, five species were identified, which were Colletotrichum camellia, Colletotrichum gloeosporioides, Colletotrichum gloeosporioides, Colletotrichum gloeosporioides, Colletotrichum gloeosporioides and Colletotrichum gloeosporioides. The morphological characteristics of the five species are described as follows.

Table 3 Morphological characteristics of Camellia oleifera anthracnose

type	Mycelial growth rate	Leaf disease spot diameter	Fruit disease spot diameter
Anthrax of Camellia sinensis	11.5±1.1b	6.4	8.3
Colletotrichum gloeosporioides	13.6±06a	1.6	1.5
Colletotrichum gloeosporioides	13.5±08a	3.5	1.5
Cryptic anthrax	12.7±06ab	1.4	1.6
Colletotrichum gloeosporioides	12.1±0.3b	1.0	2.3

About 0.5g mycelium was scraped with a sterile toothpick, and DNA was extracted with a fungal genomic DNA Extraction Kit produced by Beijing solibao Technology Co., Ltd., and stored at - 20 ℃. The intergenic regions of apn2 and mat1-2-1 genes were amplified and sequenced using am-f and am-r primers. The PCR procedure was as follows: 94 ℃ 5 min, then 95 ℃ 45 s, 56 ℃ 45 s, 72 ℃ 1 min, 30 cycles, and then extended at 72 ℃ for 7 min. PCR products were sequenced by Beijing Qingke Xinye Biotechnology Co., Ltd.The apmat sequences of 95 isolates of Colletotrichum gloeosporioides and 33 reference strains downloaded from GenBank database were analyzed for phylogenetic analysis [21]. Multiple sequence alignments were generated using Clustal w (Thompson et al., 1994) and manually edited in Mega 7 if necessary. The phylogenetic relationship was analyzed by distance method. The distance matrix was calculated by Kimura's two parameter model. The n-j phylogenetic tree of apmat gene sequence of all strains was constructed with mega 7 software. The bootstrap test was repeated 1000 times to obtain the support rate of each branch.The results of molecular variation analysis showed that the variation of pathogen population mainly occurred within the population, and the variation among populations was relatively low. The intra population variation accounted for 81% of the total variation, while the inter population variation only accounted for 19% [22]. This indicated that gene exchange between different populations of Colletotrichum gloeosporioides in Guangdong was frequent. The variation difference among and within populations of Colletotrichum gloeosporioides was significantly greater than 0, indicating that there was genetic variation in different host populations of Colletotrichum gloeosporioides ($P < 0.05$). Using ntsys PC software (version 2.1), ISSR-PCR was used to cluster the strains of Colletotrichum gloeosporioides in Guangdong Province, and the phylogenetic tree of Colletotrichum gloeosporioides was constructed, as shown in the figure.

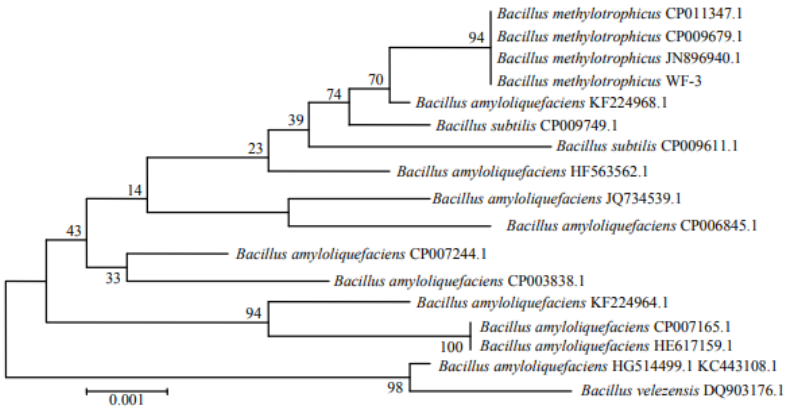


Fig. 2 Phylogenetic tree based on gene sequence of Colletotrichum gloeosporioides

95 suspected strains of Colletotrichum were isolated from 210 diseased leaf samples. 95 apmat gene sequences were obtained by sequencing. Further BLAST search in GenBank confirmed that these sequences belonged to Colletotrichum. According to Koch's rule, the 95 strains were identified as the pathogen of Camellia anthracnose. From the phylogenetic tree of Colletotrichum gloeosporioides in Camellia oleifera, 170 strains were clustered into four groups at the level of genetic similarity coefficient of 0.72. The clustering results showed that there was high genetic similarity among 170 strains of Colletotrichum gloeosporioides, but there were still some genetic variations within the species [23]. The strains from the same geographical origin were classified into different groups or subgroups. Conidia of Colletotrichum gloeosporioides are the main source of primary infection and reinfection of Camellia oleifera. Therefore, the number of forest fungal sources directly affects the severity of the disease. In addition, the growth environment of Camellia oleifera also has a certain impact on the occurrence of disease. Therefore, the breeding of Camellia oleifera with resistance to Colletotrichum gloeosporioides will promote the healthy and rapid development of Camellia oleifera industry.

5. Conclusion

At present, the research on Anthracnose of Camellia oleifera and in the prevention control in China has made good progress. Including breeding high resistant varieties, cross breeding, compound cross and so on. But there are still many problems, such as the lack of disease prediction and prediction, the uncontrolled abuse of chemical agents, the lack of disease resistance mechanism and integrated control technology, and the research on the succession law of anthracnose. Most of the Camellia oleifera planting areas are operated extensively, even in a semi natural state. The operators only take care of the fruits, regardless of tending, weeding, fertilization and pest control.At present, there is no efficient control method for Camellia oleifera disease, and chemical control is still the fastest and most effective control measures. However, with the increasing demand of consumers for tea oil quality and the change of people's consumption concept, the abuse of chemical pesticides can not effectively control the diseases and insect pests of Camellia oleifera, but also pollute the Camellia oleifera forest, reduce the quality of camellia oil and affect people's health.So in order to prevent and cure the diseases of Camellia oleifera, it is necessary to grasp its occurrence regularity and apply appropriate amount of pesticides in time. In the future, we should further strengthen the research on the biological characteristics and control methods of these diseases, and take green, efficient and sustainable control measures, which will not only escort the development of Camellia oleifera industry, but also provide a strong guarantee for China's grain and oil security.

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