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Research Article

Fungal Diseases in Rice Crops (Oryza Sativa L.) In Daule Canton, Guayas Province

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ABSTRACT

The research was carried out in the rural parishes: Laurel, Juan Aguirre Bautista, Los Lojas and Limonal, belonging to the Daule canton, province of Guayas; it is worth mentioning that the canton is known as the rice capital of Ecuador, because it covers the largest cultivated agricultural area. The main objective was to establish a diagnosis of the fungal diseases with the highest incidence at the research site. The specific objectives were: to carry out a baseline diagnosis of rice crop producers; determine fungal diseases of greater incidence with Koch's postulates; and, to make a caloric map of the farms affected by fungal diseases. The research is non-experimental, therefore, it does not have treatments, since the data were obtained by taking samples extracted in the field and analyzed in the laboratory using Koch's postulates, in order to identify the causal agent of the predominant diseases of the research sector. In the microscopic evaluation carried out in the laboratory, the presence of phytopathogens such as: Rhizoctonia solani, fusarium oxysporum and Aspergillus flavus was evidenced, resulting in the disease of sheath blight as the most predominant due to its high incidence in the study area. With the elaboration of the caloric map, the presence and incidence of fungal diseases is evidenced, with the causative agent Rhizoctonia solani being the most predominant in the study area. Constant monitoring in the field is considered one of the most effective strategies for the control of fungal diseases in the crop, since in this activity the possible effects that may develop in the different stages of the crop are evaluated.

Keywords: Sheath blight, fungal diseases, Koch's postulates, rhizoctonia solani.

INTRODUCTION

Rice (*Oryza sativa* L.) is a plant of the grass family (poaceae), native to South and East Asia, its beginnings date back to Southeast Asia from India, over time it passed to China. This cereal is one of the most remote and prevalent plant-based staples in households around the world, it is considered a valuable source of energy, in addition to being a key component of food security and economic sustainability of the population (Absalan et al., 2024).

In Ecuador, rice production began in the seventeenth century, but its consumption and commercialization were strengthened in the nineteenth century. Currently, the production of this grass reaches an area of 312.9 thousand hectares planted, where it has mainly been concentrated in the coastal region, particularly in the provinces of Guayas (65.9%), Los Ríos (28.8%) and Manabí (4.8%) where it covers 98% of the production at the national level. The Daule canton belonging to the province of Guayas is known as the rice capital of Ecuador (Orbe & Cuichán, 2022). In this locality, approximately 48852 hectares of rice cultivation are focused (El-Beltagi et al., 2024).

In rice cultivation, there is a set of diseases that reduce the percentage of productivity and yield (Duan et al., 2024). The fungal diseases with the highest incidence in the producing areas in Ecuador are: pod rot, rice burning, leaf charcoal and bacterial blight, which are caused by pathogenic fungi present in the field. It is estimated that production losses can reach up to 70 percent (Bosco et al., 2024).

Azorra (2018) indicates that the diagnosis and monitoring of crops allows the timely determination of the causal agents that cause fungal diseases, this being a recommended alternative to achieve effective control in rice cultivation, allowing the optimization of resources and counteracting the negative effects on the environment (Chowdhury et al., 2024).

MATERIALS AND METHODS

Research approach: This research focused on a descriptive analysis, the information collected was evaluated in the field and laboratory, with the aim of identifying and monitoring the diseases with the highest incidence in rice cultivation in the Daule canton (Gandikota et al., 2024).

Type of research: Desk research, Descriptive research, Field and laboratory research (Gull et al., 2024).

Research design: The research is non-experimental, since the data were obtained through monitoring in different production areas, by taking samples extracted from the field to be examined in the laboratory, in order to identify the causal agent of the main fungal diseases in rice cultivation (Hafeez et al., 2024).

Variables under study

Independent variable: Identification of fungal diseases using Koch's postulates in rice cultivation.

Dependent variable: Type of symptoms "By means of a 10X stereoscopic magnifying glass, the symptoms of the plants under study were observed, taking samples in the field for laboratory analysis." Description of the fungus "The identification of the most predominant causal agent in the area was examined in the laboratory using Koch's four postulates after the use of a microscope. According to Jena et al. (2024) to test the hypothesis that the pathogen is the cause of the disease, the following statements should be considered: 1) The pathogen must be associated with the disease in all diseased plants that are examined; 2) The pathogen must be isolated and develop in a pure culture (non-obligate parasite) allowing it to develop on a susceptible host plant (obligate parasite) and evidencing its presence and the effects it produces; 3) The pathogen that develops in a pure crop must be inoculated in healthy plants of the same species in which it found the disease and must develop the same symptoms of the disease in the inoculated plants; 4) The pathogen must be isolated once again in a pure culture and its characteristics must be similar to the second statement." Baseline diagnosis "a baseline diagnosis was made through a descriptive closed-response survey prepared by the author to determine factors such as: soil type, water management and disease control (types of fungicides, dose and frequency) of each study area". Analysis of samples in the laboratory "With the samples collected in the field, a laboratory

isolation of the main fungal pathogens was carried out using Koch's postulates, to inoculate them in Petri dishes with healthy rice plants, with the aim of evaluating the development of infection of fungal pathogens. After the development of inoculation, they will be infected again in healthy plant culture media to evidence the manifestation and development of symptoms, in order to verify that the healthy plant presents similar characteristics of the fungal pathogen under study." Caloric map of the farms affected by fungal diseases "By means of a navigator (GPS), the geographical coordinates of the different study areas were obtained, for subsequent elaboration of the caloric map through a geographic information system (QGIS) of the farms located in the four parishes (Laurel, Juan Bautista Aguirre, Limonal and Las Lojas) associated with the agricultural center of the Daule canton to evidence fungal diseases of the crop of rice in the area (Muthu Narayanan et al., 2024).
Statistical analysis: A descriptive analysis was used, collecting ten random samples of rice seedlings for each study farm. They were later examined in the laboratory using Koch's postulates. With the data obtained, the identification of each disease was carried out by developing a database in the Microsoft Office Excel spreadsheet for the identification of the main causal agent of the crop and a caloric map was developed using the geographic information system to determine the farms affected by fungal diseases in the canton (John & Ray, 2024).

Techniques

Collection of samples of rice plants: Samples of rice plants in the maturation stage were taken in the rice production lands belonging to the farmers associated with the agricultural center of the Daule canton. The management of the sampling was carried out randomly, going through the entire production area evaluating the plants that presented visible symptoms with characteristics similar and relevant to rice diseases. After this activity, cuts were made from the base of the stem with latex gloves and metal-tipped stylets disinfected with alcohol and sodium hypochlorite as a protective measure to avoid contamination. The collected samples were stored in plastic bags with hermetically sealed to protect them from possible physical deterioration during transport and humidity to prevent the development of fungi and bacteria, in turn they were correctly labeled with identification information to determine: date of collection, name of the owner of the production area and the description of the visible symptoms and affected parts in the rice plant, to then be transported and examined more easily in the laboratory of the Agrarian University of Ecuador "UAE".
Cleaning and disinfection of laboratory material: After the sample collection activity, the area, materials and tools of the laboratory of the Agrarian University of Ecuador were disinfected, with a homogeneous mixture of 5.25% sodium hypochlorite, 20% alcohol, vinegar and soap. All glass materials were washed with a solution composed of water, commercial detergent solution and alcohol with the help of a fine-bristled brush to remove dirt. It is important to mention that after using drinking water, rinsing with distilled water is required to remove particles and impurities present in the drinking water. They were also sterilized in an autoclave equipment because this machine provides a heating effect that generates heat, which causes microorganisms to be eliminated by water vapor and high temperatures that can reach 121 degrees Celsius, so caution is required when using it (Senapati et al., 2024).
Preparation of culture medium for the planting of pathogens: a) With the help of the electronic scale, 39 grams of PDA were weighed to be mixed in a beaker with a liter of distilled water; b) Beat the solution constantly with a metal spoon to avoid sedimentation and lumps; c) The solution obtained is heated in a heating oven to 216 degrees, stirring constantly with a metal spoon for a period of 20 minutes to achieve its dissolution; (d) This solution was sterilized in the autoclave equipment at a temperature of 121°C for a period of 25 minutes; e) After this step, the solution was allowed to rest and cool inside the autoclave to be applied in the plastic Petri dishes, it is important that this application is carried out inside the laminar flow chamber to avoid contamination; f) For a period of 2 years, it was verified that the Petri dishes did not present any type of deterioration or contamination (Suddal et al., 2024).
Cutting and disinfection of plant samples in the laboratory: The plant samples were cut with a scalpel based on the parts where symptoms of diseases were present in the plant to a length of about 12 mm, after making the cuts, before being transferred to the laminar flow chamber they were immersed in concentrations of alcohol and distilled water. It should be noted that this process was carried out with

personal protective equipment and laboratory elements disinfected to avoid contamination (Supriya et al., 2024).

Sowing of the explants in the culture medium: Once the disinfection of the plant samples has been carried out, the pathogens are seeded in the culture medium, it is important to mention that this procedure was carried out inside the laminar flow chamber. In each Petri dish, three to four portions of tissues were explanted to achieve the development of pathogens for a period of 15 days. Finally, the Petri dishes were covered with plastic wrap and sealed in plastic bags with hermetic closure to be stored in the laboratory incubator to fulfill the function of storing and generating the appropriate environment (temperature and humidity) for the development of microbiological cultures and to be protected from any contaminating material or prevent the entry of unwanted microorganisms (Wang et al., 2024).

Evaluation and seeding of pathogens in new Petri dishes: After 15 days of storage of the Petri dishes in the incubator, the first evaluation of development was carried out, in order to visualize the development of fungal pathogens through the microscope. To carry out this activity, the Petri dishes with the contaminated material were transferred to the laminar flow chamber to be discovered and avoid contamination by other pathogens present in the laboratory environment. The microscope was used, considering all the biosafety standards of the laboratory, for this activity instruments such as the object holder and cover, tape, distilled water, syringe and to be able to extract part of the contaminated material carefully to the slides and be more easily visualized in the microscope. Once the pathogens were identified under the microscope with the objectives: 4x, 10x, the sample was collected by rubbing or scraping with the bacteriological loop in the affected area and they were inoculated into new Petri dishes with PDA, in order to comply with Koch's third and fourth postulates, in which the pathogen must be isolated once again in a pure culture medium and must present similar characteristics of fungal diseases under study. Once seeded in the new culture media, they must be sealed with plastic film and kept in the incubator for a period of 15 days.

Evaluation and inoculation of pathogens: After 15 days, the second observation of the Petri dishes was made, visualizing through the microscope to verify and check if the fungal pathogens identified in the first samples planted in the Petri dishes presented the same characteristics, resulting in the identification of the main fungal diseases in rice cultivation in the Daule canton (Zheng et al., 2024).

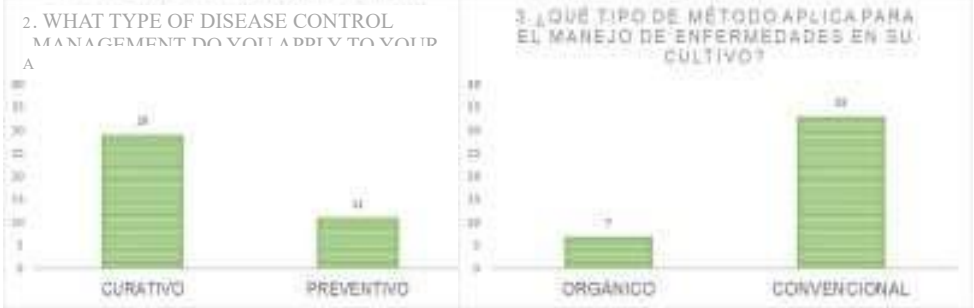
Caloric map of the main fungal diseases: A caloric map was designed in the QGIS program with data obtained in the field and laboratory, in which the main fungal diseases present in four parishes of the Daule canton were identified, such as Laurel, Juan Bautista Aguirre, Los Lojas and Limonal, using a colored symbology to identify the severity of the diseases under study. the graphic representation is based on thermography to determine the impact on these areas (Nieto-Cañarte et al., 2023).

RESULT AND DISCUSSION

Baseline diagnosis of rice crop producers

The baseline diagnosis was developed through a closed survey to determine and provide information about the agricultural management carried out by rice producers in parishes such as: Laurel, Limonal, Juan Aguirre Bautista and Los Lojas belonging to the Daule canton. With the formulation of this survey, it was possible to determine that one of the main limiting factors of production of this grass in terms of the management and control of fungal diseases is the lack of technical knowledge on the part of farmers.

Figure 1. Surveys of producers in the canton of Daule. In original language Spanish



The figure above shows that producers in the study parishes continue to use methods and empirical work for phytosanitary control. Most of the respondents answered that the type of management for the control of fungal diseases is curative, that is, they do not use preventive treatments and apply them once the plant presents significant deterioration, the type of application method is conventionally chemical, as a result they cause the causal agents not to be controlled in time with preventive management and efficient strategies, therefore, fungal pathogens maintain a high rate of spread and tend to develop a fungicide resistance effect, causing significant damage and permanence in the crop.

In relation to question six of the survey, it was determined that there is a high percentage of ignorance on the part of farmers regarding the accurate diagnosis and identification of fungal diseases present in the crop by not recognizing the symptoms and damage caused by phytopathogens, so they cannot carry out strategies to control these pathogens in the recommended time and in turn the vast majority of farmers are not able to control these pathogens. respondents do not choose to carry out control monitoring in the different stages of the crop.

Figure 2. Survey Statistics. In original language Spanish

Determine fungal diseases with higher incidence with Koch's postulates

Using Koch's theory of five postulates, samples of plant material from the areas of the study canton were evaluated in the laboratory. In the microscopic evaluation, the identification of the following pathogens was obtained: Rizhoctonia solani, Fusarium spp, these are considered as the fungal diseases with the highest incidence of the rice-producing areas of the Daule canton, while the phytopathogenic fungus with the lowest percentage of manifestation was Aspergillus Flavus.

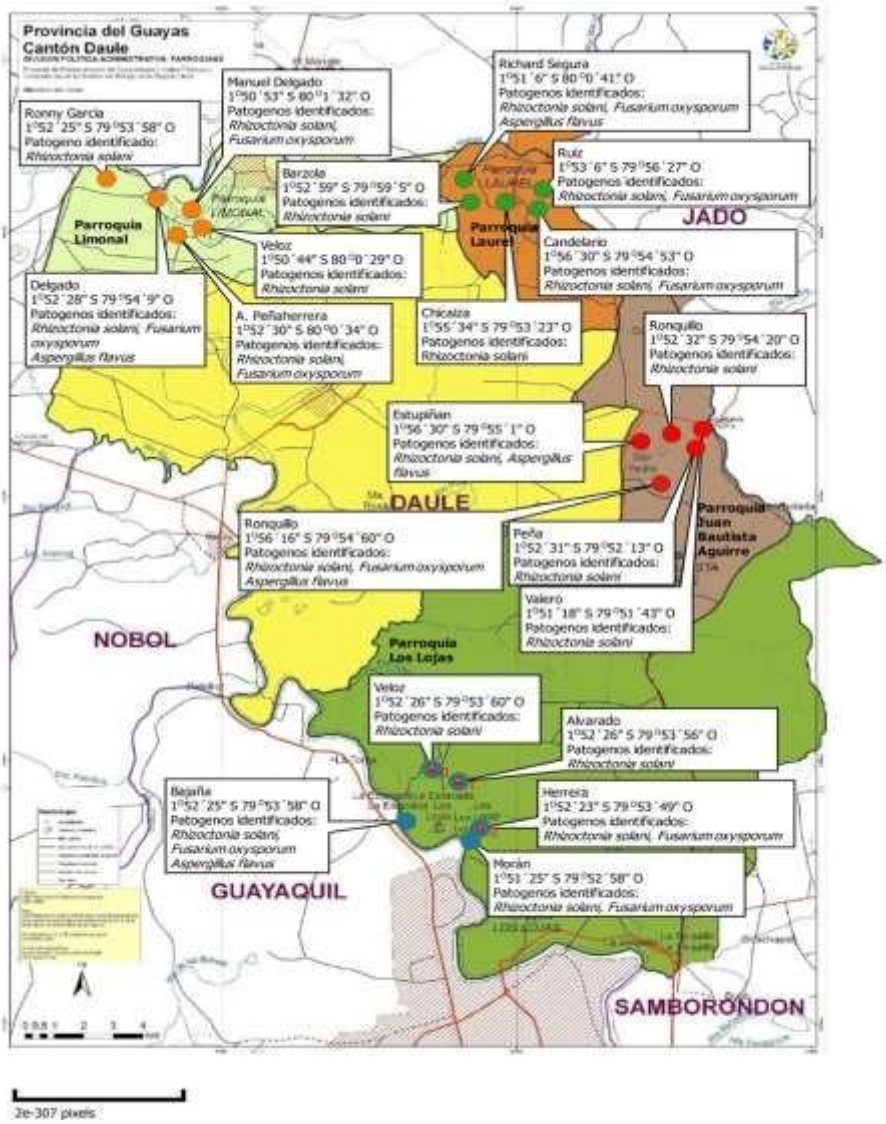
In the microscopic examination, colonies formed by septate hyphae of dark brown pigmentation with long ramifications at right angles in relation to the main hyphae were visualized, near the point of formation of the hyphae the presence of dorlipores septa can be observed considering dividing or transverse walls. Due to the similar characteristics of the pathogen, it is shown that it is the causative agent Rhizoctonia solani. It can also be noted that the development of this fungus in the boxes with PDA culture medium showed creamy white mycelial growth with brownish brown spots. As for other microscopic characteristics, it was observed that in different boxes of culture medium the presence of the phytopathogen Fusarium spp is evidenced, when identifying elongated macro conidia in oval, cylindrical or crescent clusters, these also present septate hyphae and chlamydospores. Macroscopically, this pathogenic fungus develops a colony of beige mycelia with a spongy characteristic, which over the days isolated in the Petri dishes produces a wine-colored pigmentation. Finally, the pathogenic fungus that occurred with the lowest incidence is Aspergillus flavus, microscopically recognized for having thick-walled branched conidiophores and the conidia are smooth, rough and radiated spherical in shape. While in the macroscopic evaluation it is visualized that the colonies initially present a whitish mycelium coloration of cottony texture that over time manages to acquire a powdery yellowish-green coloration, occasionally they form in the center or margin of the colony. It should be noted that this phytopathogen can develop fast-growing colonies in the PDA Petri dish.

Caloric map of farms affected by fungal diseases

Fungal diseases considerably affect agricultural production in the country, in the Daule canton the agricultural production with the highest incidence is the cultivation of rice, for this reason this Guayasense canton is known as the rice capital of Ecuador, however, in recent years the crop has been affected by the presence of fungal diseases that harm yield and cause considerable losses to producers and/or farmers.

Figure 3 shows the geo-referential map of the parishes: Laurel, Limonal, Juan Bautista Aguirre and Los Lojas belonging to the Daule canton, where the determination and identification of the main fungal diseases present in rice cultivation in those areas was carried out, in addition, the coordinates, data of the owners of the evaluated plots and the main diseases that were found in the production areas are detailed. the map was generated by using the QGIS program.

Figure 3. Identification of fungal diseases found in the canton of Daule. In original language Spanish

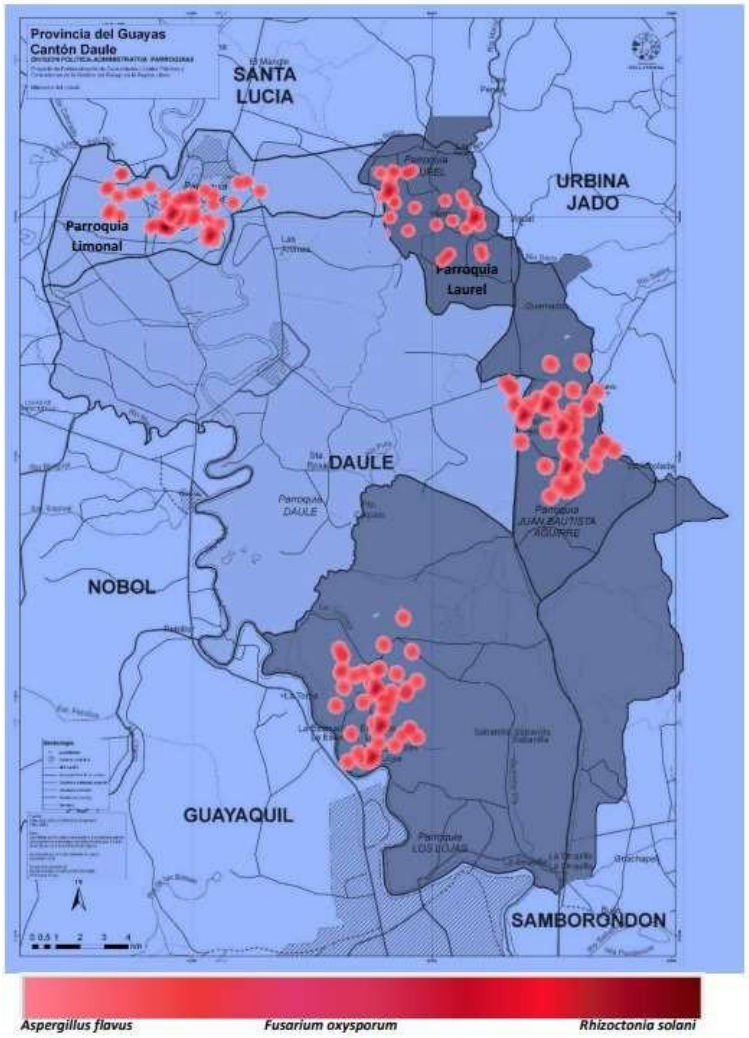


According to Vélez-Ruiz et al. (2024), it determines through Koch's postulates the identification of fungal diseases, in which he found that the phytopathogens present in rice growing areas are the burning caused by the causal agent Pyricularia oryzae, rice blight caused by the pathogen Rhizoctonia solani and the bacterial blight caused by the pathogen Burkholderia glumae, considering the burn disease as the most predominant in the area in terms of of study. Meanwhile, Tigrero-Zapata et al.

(2022) indicate that in their research work, the disease with the highest incidence in rice cultivation was sheath blight caused by the phytopathogenic fungus Rhizoctonia solani, causing significant damage in production areas.

On the other hand, Figure 4 shows the caloric map generated through the use of the QGIS program, where the intensity of the presence of fungal diseases is observed, with *Rhizoctonia solani* leading the list of species with the highest intensity, followed by *Fusarium oxysporum* and *Aspergillus flavus*, the latter being the one with the lowest presence in the rice crops evaluated.

Figure 4. Calorie map of major fungal diseases found in rice. In original language Spanish



It is established that the use of heat maps through the information system can collect base information of the areas or farms affected by the presence and attack of diseases in the rice crop, this allows the producer to have knowledge of the pathogens present in the production areas. Nieto-Cañarte et al., (2023) demonstrates the incidence of the pathogen in different study lots through heat maps prepared with GIS software (QGIS). Likewise, Tigrero-Zapata et al. (2022) present, through the use of thermography in heat maps, the evaluation of damage caused and the location of the presence of phytopathogenic fungi in the experimental areas.

CONCLUSIONS AND RECOMMENDATIONS

In the surveys carried out on producers in the area, it is determined that there is a lack of technical knowledge regarding the phytosanitary control of rice cultivation, resulting in ineffective and incorrect management and control for the control of fungal diseases.

Using Koch's postulates, it was determined that the samples collected in rice production areas of the Daule canton and examined in the laboratory of the Agrarian University of Ecuador (UAE), presented a higher percentage of fungal diseases, characteristic of the area such as: *Rhizoctonia solani*, *Fusarium oxysporum* with a lower percentage *Aspergilluss flavus* causing effects on the vegetation and maturation stage of the rice crop.

Constant monitoring in the field is considered one of the most effective strategies for the control of fungal diseases in the crop, since in this activity the possible effects that may develop through the presence of the different pathogens present in the crop fields in the different phenological phases of the crop are evaluated.

The caloric map developed in this research shows that the parishes belonging to the Daule canton, such as "Juan Bautista Aguirre" and "Los Lojas", have a high rate of areas affected by the main fungal diseases of rice cultivation.

Based on the research carried out, it is recommended: Implement selective sampling standards in the activity of taking and handling samples in the field. Encourage rice producers to be part of agricultural organizations or groups that provide technical training in agriculture in order to expand knowledge of the crop. Apply cleaning and disinfection procedures in laboratory activities to avoid contamination of the environment. Carry out field monitoring in each of the phenological stages of the crop to identify possible effects caused by pathogens. To use Koch's postulates for future research on the identification of fungal pathogens in rice cultivation. Share the study information with the producers in the area, to determine the importance of phytosanitary control and management of rice cultivation, to avoid production losses.

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