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Effectiveness of Extract of Jeringau Rhizome (Acoruscalamus L.) on Mushrooms Sclerotium rolfsii Sacc. Causing Stem Rot on Peanut Plant (Arachis hypogaea L.)

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ABSTRACT

SclerotiumrolfsiiSacc. is one of the important pathogens in peanuts. The controls that have been undertaken by farmers generally use synthetic pesticides. However, the use of synthetic pesticides has various negative impacts on the environment and human health. Therefore a technology is needed to produce environmentally friendly pesticides and effectively control stem rot. This study aims to determine the effectiveness of the Jeringau rhizome extract against S. Rolfsii fungi. The results showed that the crude extract of the Jeringau rhizome was able to inhibit the growth of S. rolfsii fungi in vitro on PDA media. Minimum Inhibitory Concentration (MIC) crude extract of jeringau rhizome was 0.50%. The inhibitory power of the extract with a concentration of 0.10% to 0.50% increased from 48.17% to 100%. The treatment of extract concentration of 0.50% showed fungicidal properties against S. rolfsii.

Keywords: Acoruscalamus L., stem rot, Sclerotiumrolfsii.

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1. INTRODUCTION

1.1. Background

SclerotiumrolfsiiSacc. is a pathogen that causes stem rot in peanutplants. This fungus is polyphagic, which can attack various plants (Sunardi, 1988). This fungus is more difficult to control because of its wide host range, rapid growth, and the ability to produce sclerotia which can survive as saprophytes in the soil or in plant residues. Until now, pathogenic fungi that cause stem rot in peanutplants are still a crucial problem and there are no peanutvarieties that are truly resistant to the disease (Astiko et al., 2009).

The controls that have been carried out so far are the use of synthetic pesticides. According to Suprapta (2003) the use of synthetic pesticides may have a negative effect on the ecosystem, due to the accumulation of active chemicals that are difficult to decompose by microorganisms in nature. The negative effects of synthetic pesticides also affect humans.

There are many negative impacts of synthetic pesticides certainly require alternative controls that are more environmentally friendly. The use of vegetative pesticides is an alternative control that can be applied. According to Novizan (2002) the advantages of vegetable pesticides are: a) Vegetable pesticide residues are more quickly decomposed by natural components, thereby reducing the risk of water and soil pollution, b) Vegetative pesticides have relatively fast action compared to natural pesticides and biological pesticides because they work fast in stopping pest appetite, or preventing more destructive pests, c) high selectivity so that the negative impact on beneficial organisms is very small, d) the way it works is different from synthetic pesticides, so it can be

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relied upon to overcome pests that have been resistant to synthetic pesticides, e) low phytotoxicity, where vegetative pesticides do not poison and do not damage plants, f) toxicity (toxicity) is generally low against mammals, making it relatively safer for humans and livestock.

The handling of various plant diseases by using natural ingredients continues to be carried out, one of which is the use of environmentally friendly bushes. Jeringau is one example of a plant that may also be used as a vegetative pesticide. The part of the plant that is often used is part of the rhizome. The use of jeringau rhizome can inhibit the growth of Escherichia coli and Vibrio cholerae. In general, the two bacteria tested could be inhibited by jeringau rhizome extract with the largest inhibitory diameter being 12.1 mm and 12.4 mm respectively for E. coli and V. cholerae (Antara et al., 2008).

Besides being able to inhibit bacteria, the jeringau rhizome is also able to inhibit the growth of fungi. The jeringau rhizome extract was able to inhibit the growth of fungi Botryodiplodiat heobromae which causes banana rot disease (Rustini, 2004). To enrich the information about the benefits of jeringau rhizomes, their effectiveness in inhibiting the growth of S. rolfsii fungi was investigated. The use of jeringau rhizomes in inhibiting the growth of S. rolfsii fungi has never been done, therefore it is necessary to do research on the effectiveness and potential of jeringau rhizomes to control S. rolfsii fungi in vitro.

1.2. Problem Formulatiosn

1. Is the jeringau rhizome extract able to inhibit the growth of S. rolfsii fungus which causes stem rot in peanuts?
2. What is the minimum concentration of jeringau rhizome extract that can inhibit S. rolfsii's growth?

1.3. Research Objectives

1. Finding out the inhibitory power of jeringau rhizome extract on S. rolfsii fungus growth causing stem rot in peanuts in vitro.
2. Finding out the minimum concentration of jeringau rhizome extract which can inhibit the growth of S. rolfsii fungi.

1.4. Research Significance

After finding out the inhibitory power and minimum concentration of jeringau rhizome extract on S. rolfsii fungus growth, these results can be used as one of the integrated control components of S. rolfsii fungus which causes stem rot disease in environmentally friendly peanuts.

2. MATERIALS

2.1. Peanuts (Arachishypogaea L.)

Peanuts are food crops in the form of shrubs originating in South America, precisely from Brazil. Peanuts first entered Indonesia in the early 17th century, brought by Chinese and Portuguese traders (Soesanto, 2004). Peanuts are one of the commodities that have economic significance, because in addition to producing oil and as a snack, it is also rich in fat (40.50%), high protein, iron, vitamins (A, B, C, D, E and K), carbohydrates, and minerals including Calcium, Chloride, Ferro, Magnesium, Phosphorus, Potassium and Sulfur (Hardaningsih et al., 2007).

According to Astiko (2009), peanuts are a type of tropical plant that grows in shrubs as high as 30 to 50 cm and has small leaves. This plant is the second most important plant after soypeanuts in Indonesia.

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2.1.1. Peanut Morphology

According to Rukmana (1998) the classification of peanut plants is as follows:

Kingdom : Plantae
Divisio : Spermatophyta
Subdivisio : Angiospermae
Class : Dicotyledone
Ordo : Leguminales
Family : Papilionaceae
Genus : Arachis
Species : Arachidishypogeae L.

Stems of peanut plants are short, a type of upright or horizontal growth (Fig. 1). Joins or stem segments located in the ground are places attached to roots, flowers, and fruit. The stem segments that are on the ground are where the stems grow. The leaves are oval-shaped, located in pairs (compound), and even-finned. Each leaf stalk consists of four leaflets. Leaf strands are nititropic, which is possible to absorb. The surface of the leaf has a meaning that is as a barrier or storage of dust (Soesanto, 2004).

Fig. 1 Peanut Plant

1.3. Sclerotium rolfsii fungus Causes stem rot in peanuts

Stem rot caused by sclerotium rolfsii is a common disease in peanut plants (Semangun, 2000). This disease is often also called sclerotium rot, and if affected it can cause severe damage. This is one of the inhibiting factors for increasing peanut production in Indonesia (Pudjihartati et al., 2006; Hardaningsih and Hadi, 2007).

S. rolfsii's infection in peanut cultivation results in a decrease in yield in quality and quantity. Quantitatively, S. rolfsii infection in vulnerable peanut plants in the field can reduce yield by up to 80% (Widyanti, 2001; Rani, 2001). Peanut varieties or genotypes in Indonesia have reportedly not been resistant to this fungal infection. The test results in a controlled environment showed that 30 tested genotypes of peanuts were rather vulnerable (4 genotypes), susceptible (11 genotypes), and very susceptible (15 genotypes) to S. rolfsii infection, and none were resistant or somewhat resistant (Yusnita and Sudarsono, 2004).

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Fig. 2. Peanut Stems Infected with S. rolfsii's Fungu

2.6. Symptoms of stem rot

Symptoms of plants affected by S. rolfsii begin with the appearance of lesio on a stem covered with white mycelia (Fig. 2). Lesio is widening over time and is accompanied by decay of the stem surface. The broadening of lesio in the stem is followed by the change in the color of the stem at the lesio edge from light brown to dark brown. Starting on the 10th day, brownish white sclerotia spots begin to form on the surface of the decaying stem. After stem decay extends, and sclerotia formation from fungal hyphae occurs, some leaves in the main branch begin to wilt and then infected plants die (Melouk and Backman, 1995).

2.4. Sclerotium rolfsii Morphology

According to Alexopoulos and Mims (1979) S. rolfsii mushrooms can be classified as follows:

Kingdom : Mycetae
Devisio : Amastigomycota
Sub divisio : Deuteromycotina
Class : Deuteromycetes
Sub Class : Deuteromycetidae
Ordo : Agronomycetales
Family : Agronomycetaceae
Genus : Sclerotium
Species : Sclerotium rolfsii Sacc.

The S. rolfsii fungus grows optimally at 27-300 C, with the optimum pH range for mycelium is 3.0-5.0, and germination of sclerotia occurs between pH 2.0-5.0 (Punja and Grogan, 1981), and is inhibited at pH above 7.0 (Sharma and Kaushal, 1979). This fungus is very suitable to develop in sandy soil and low nitrogen content. Sclerotia is not active at temperatures below 00C (Punja and Rahe, 2001).

2.1. How Sclerotiumrolfsii fungal infected

Sclerotiumrolfsii firstly attacks the stem, especially the base of the stem, which is a soft part of the plant that causes the base of the stem to rot so that the disease is often referred to as stem rot or sclerotium rot (Ferreira & Boley, 2006). In addition to attacking the S. rolfsii stem, it also infects several parts of the plant in very humid conditions, including roots, fruit, leaves and flowers. The initial infection by the fungus occurs on the ground surface, where sclerotia will germinate and before penetrating the host tissue will produce mycelium. Host tissue penetration occurs when the fungus secretes an enzyme that damages the outer layer of the host cell (Punja and Rahe, 2001).

When infecting peanuts, S. rolfsii releases large amounts of oxalic acid toxins, which can kill the epidermal cells

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of the host plant (Melouk and Backman, 1995). In addition, a number of enzymes that function to increase the permeability of host plant cells are also secreted by S. rolfsii, causing electrolyte leakage of cells / tissues of affected plants.

In the pathogenesis process, S. rolfsii synthesizes and secretes large amounts of oxalic acid (OA) phytotoxins (milimolar concentrations) and cell wall degrading enzymes such as endo-polygakturonase (endo-PG) and cellulase into infected tissue. OA causes acid pH in plant tissue, making it optimum for endo-PG enzyme activity. In addition OA is able to bind C ++ from the cell wall, so that the cells and tissues of the host plant are very vulnerable to cell wall degrading enzymes released by the fungus (Semangun, 2000). Plants that are 2-3 weeks old are most susceptible to S. rolfsii (Semangun, 1993).

2.3. Pesticide

According to The United States Environmental Pesticide Control Act, (Munaf, 2007) pesticides are

1. All substances or mixtures of substances that are specifically used to control, prevent, or fend off disorders of insects, rodents, nematodes, weeds, viruses, bacteria, microorganisms that are considered pests, except for viruses, bacteria, or other microorganisms found in humans and animals.
2. All substances or mixtures of substances specifically used to manage plant growth or plant dryer for major plant disruptors known as herbicides.

Pesticides are toxic materials so that if their use is unwise it can cause negative impacts, both directly and indirectly for human health and the environment (Kardinan, 2004). Kardinan (2004) further describes the adverse effects of pesticides, among others: (1) heavy metals which are elements of pesticides are usually deposited in the liver, thus affecting metabolism and causing damage to the kidneys; (2) pesticides can also interfere with the circulation of hormones which cause testicular effects and cause a number of diseases such as prostate cancer, female reproductive problems, breast cancer, and behavior changes; (3) disturbing aquatic life, for example killing fish.

2.4. Vegetative Pesticides

Vegetative pesticides are pesticides that use plant secondary metabolites as raw materials (Wiratno, 2010). Nature has actually provided natural ingredients that can be used to overcome pests and plant diseases, as a substitute for chemical pesticides. Kardinan (1998) stated that the use of vegetable pesticides is not intended to abandon synthetic pesticides, but is merely an alternative to minimize the use of synthetic pesticides so that environmental damage can be minimized.

The ability of plants in controlling various diseases is inseparable from the content of organic compounds. Phytochemical or plant chemistry studies various kinds of organic compounds that are formed and stockpiled by plants, namely regarding their chemical structure, biosynthesis, changes and metabolism, their natural distribution and biological functions (Harborne, 1996). Plants produce various types of abundant organic compounds, most of which do not appear directly in the growth and development of these plants. These chemicals are simply referred to as secondary metabolites whose existence is limited to certain species in the plant kingdom. Secondary metabolites are

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also known as natural metabolic results (Croteau et al., 1994).

2.5. Jeringau (Acorus calamus L.)

According to Kardinan (2004), Jeringau can be classified as follows:

Divisi : Spermatophyta
Sub divisi : Angiospermae
Class : Monocotyledonae
Ordo : Arales
Family : Araceae
Genus : Acorus
Species : Acorus calamus L.

Jeringau is a type of medicinal plant that lives on relatively moist to runny soil. Jeringau is a perennial herb with a height of about 75 cm. This plant usually lives in a humid place, such as swamps and water at all altitudes. The stem is wet, short, forms a rhizome, and is dirty white. The leaves have strong leaf bones, are located in the middle with pointed tip leaves, flat edges, 60 cm long, about 5 cm wide, and dark green. The flowers are small, greenish yellow and smell very fragrant. Tapered tip, 20-25 cm long, is located on the leaf's leaf (Wijayakusuma, 2001).

3. METHODS

3.3. Research Conceptual Framework

Peanuts (Arachishypogaea L.) are leguminous plants belonging to the Papilionaceae family, the second most important plant after soypeanuts in Indonesia. Peanutplants can be used for animal feed, while the seeds are used as a source of vegetable protein, oil and others. Peanuts Productivity can decrease due to attacks of various diseases. The development of plant diseases depends on three components of the disease, namely pathogens, hosts, and the environment. If one component does not support, then the disease will not occur (Abadi, 2003). There are many diseases that can attack peanutplants, one of which is stem rot caused by the S. rolfsii fungus. Suitable environmental conditions and susceptible hosts to these pathogens can cause significant diseases.

Disease control with synthetic fungicides by farmers has been less effective in controlling diseases, and has caused various problems that are detrimental to human life whether directly or indirectly, including causing residues that are attached to plant products that will disrupt consumer health, environmental pollution, and kill other organisms who is not a target. There are other alternatives that may be used as pesticides, namely by utilizing plants, which are known as vegetative pesticides. One that can be utilized is the jeringau rhizome which is commonly used as spices. This study tested the activity of extracts and active compounds in the jeringau rhizome which are effective as vegetative pesticides.

3.2. Research Location and Time

This research was conducted at the Biopesticide Laboratory of the Faculty of Agriculture, Udayana University, Analytical Chemistry Laboratory,Udayana University.

3.3. Materials and Tools

The materials in this study were crude extract of jeringau rhizome, S. rolfsii mushroom, PDA (Potato Dextrose Agar) media, PD Broth media (Potato Dektrosa broth), peanutstem, aquades, microscope, 70% alcohol, hexane, dichloromethane, ethyl- acetate, methanol, acetone, sterile water containing 10% tween-80, and silica gel with a particle size of 75-150 µm. The tools used in this study are laminar flow cabinet, bunsen lamp, cutting knife, sprayer, knife, aluminum foil, measuring cup, test tube, petri, needle ose, scales, erlenmeyer, glass alarm, cork borer, label

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paper, autoclave, micropipette, tissue, tweezers, gauze, filter paper, stirring spoon, 2 kg plastic, vacuum rotary evaporator, label paper, separating funnel, gas stove, pan, stirring spoon, pumpkin mixer, and ruler.

3.4. Extraction Method

The dried rhizome used in this study was obtained from Beng Village Gianyar, the active ingredient was extracted. Extraction is carried out by chopping small pieces of dried rhizome. The chopped results are dried for 2-3 days. The dried jeringau rhizome is macerated in methanol with a ratio of 1:10 (weight / volume) for 48 hours with the aim of attracting the active ingredient in the material to be used as vegetable pesticides. The filtrate obtained by filtering through gauze was then evaporated using a vaccum rotary evaporator at 400C, so that a crude extract was obtained.

The crude extract was weighed, recorded its weight and calibrated with the weight of methanol in the same volume as the crude extract of the rhizome rhizome to determine the initial concentration. Dilution of the extract was carried out by adding tween-80 10% water as the solvent.

3.1. Antifungal Activity Test with Diffusion Well Method

The test was carried out by testing the antifungal activity of crude extract of the jeringau rhizome against S. rolfsii. Petri dishes containing 10 ml of PDA media and 1 ml of S. rolfsii were allowed to solidify. After the solid diffusion wells were made 2 pieces in each Petri dish using a cork borer. Each diffusion well is filled with 20 µl of crude extract. According to Ardiansyah (2005), if the resistance zone is ≥ 20 mm (very strong inhibition), 10-20 mm (strong inhibition), 5-10 mm (medium resistance), and <5 mm (less or weak inhibition) .

Tests to determine Minimum Inhibitory Concentration (MIC) were also carried out by the diffusion well method with several extract concentrations, namely: 0%; 0.25%; 0.5%; 0.75%; 1%; 2%; 3%; 4% and 5%. The solvent which is used is sterile water containing 10% tween 80.

3.6. Antifungal Activity Test on the Growth of Mushroom Colonies

Testing of antifungal activity against the growth of fungal colonies using several extract concentrations, namely: 0%, 0.1%; 0.2%; 0.3%; 0.4%; 0.5%. Each concentration was made 1 ml then mixed with 10 ml PDA liquid. Wait for a while until the PDA mixture and extract solidified then S. rolfsii fungus isolates which had been cultured on Petri dishes were taken and separated using a 5 mm diameter cork, then using a isolate ose needle the fungus was placed right in the middle of the Petri dish. Each extract concentration was made with three (3) replications. Mushroom culture without extract was prepared as a control. Then it was incubated at room temperature for several days until the fungus in the control filled the Petri dish.

4. RESULTS AND DISCUSSION

4.1. Sclerotium rolfsii Morphology

Sclerotiumrolfsii mushroom is an important pathogen because it has a wide host range and the ability to form sclerotia. S. rolfsii has a mycelium consisting of white threads, arranged like feathers or fans. This fungus does not form spores but forms a number of sclerotia which function for dispersing and defending themselves. Sclerotia is initially white, then brown, with a diameter of approximately 1 mm.

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These sclerotia particles are easily released and transported by water (Semangun, 2000). The form of sclerotia is presented in Fig. 3.

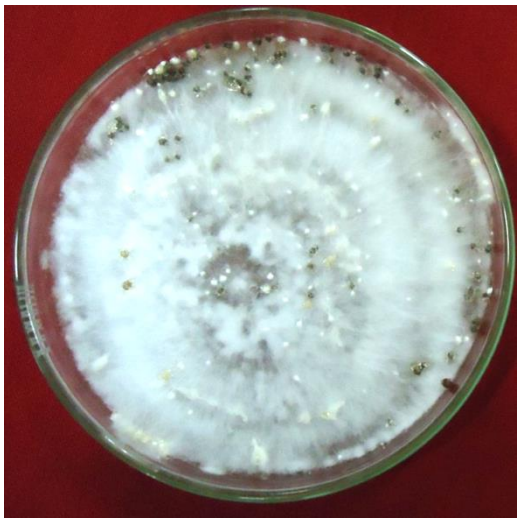

Fig. 3. Mushroom growth of S. rolfsii On PDA media

4.2. Antifungal Activity of Crude Extract of JeringauRhizome on PDA Media

Based on the results of the antifungal activity test, it was shown that the crude extract of the jeringau rhizome was able to suppress the growth of S. rolfsii fungi on PDA media. Based on the measurement results, the diameter of the inhibition zone formed is 26 mm. According to Ardiansyah (2005), if the diameter of the inhibition zone is 5 mm or less, the inhibitory activity is categorized as weak, if the 5-10 mm inhibition zone is categorized as moderate, 10-19 mm is categorized as strong, and 20 mm or more is very strong. So that the jeringau rhizome which has a diameter of inhibitory zone of 26 mm including has a very strong inhibitory power against S. rolfsii fungi. The antifungal activity of the crude extract of jeringaurhizome is shown in Fig. 4. The inhibitory power of the crude extract ofjeringau rhizome in various concentrations is presented in Table 1. The test with diffusion well method showed that the Minimum Inhibitory Concentration (MIC) crude extract of the jeringau rhizome on the growth of S. rolfsii fungi was 0.5% with a barrier zone of 8.70 mm on the third day of incubation.

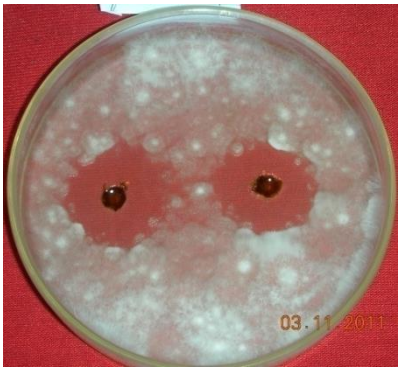

Fig. 4. Activity of inhibitory power of Crude Extract of JeringauRhizome on S. rolfsii on PDA Media after 3 days of incubation

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Table 1
Antifungal Activity of Some Concentrations of Crude Extract of JeringauRhizome on PDA Media

Extract Concentration (%)	Resistance zone diameter (mm)
0	0
0,25	0
0,5*	8,70
0,75	10,50
1	15,40
2	18,33
3	21,17
4	23,50
5	24,00

Note (*): Minimum Inhibitory Concentracion (MIC)

Referring table 1 it can be seen that the higher the concentration of crude extract of the jeringau rhizome given to PDA media, the greater the inhibition of S. rolfsii growth. According to Mustika and Rachmat (1993), the concentration of a material that functions as an antimicrobial is one of the small determinants of its ability to inhibit the growth of microbes tested.

4.3. The Effect of Extracts on the Growth of Mushroom Colonies on PDAs

Quantitatively, the crude extract of the jeringau rhizome was able to inhibit the growth of S. rolfsii fungal colonies on PDA media, although S. rolfsii was one of the strongest fungi in the soil. The resistance of the crude extract of the jeringau rhizome to the S. rolfsii fungus is presented in Table 2. The higher the concentration of extract mixed with the media, the smaller the growth of S. rolfsii fungus colonies or the inhibitory power of the jeringau rhizome extract to the growth of S. rolfsii mushroom colonies was greater. The concentration of 0.4% jeringau rhizome extract showed inhibition of 91.83% while the inhibitory power of 100% in the growth of S. Rolfsii fungal colonies occurred at a concentration of 0.5%. These data indicate a tendency that an increase in extract concentration was followed by an increase in inhibition of S. rolfsii. The treatment of various concentrations of jeringau rhizome extract against S. rolfsii colony growth is presented in Fig. 5. The inhibitory ability of the jeringau rhizome extract against S. rolfsii was due to the presence of antifungal active compounds against S. rolfsii. Sudana (2004), states that the high and low antimicroorganism activity of a compound is determined by the chemical properties of the compound such as the shape and length of the compound chain, ability to penetrate the cell wall, molecular integrity in cells and hydrophilic or lipophilic properties of a compound.

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Table 2. Inhibition of Several Concentrations of crude extract of jeringau rhizome on S. rolfsii

Extract Concentration (%)	Average colony diameter(mm)
0,0	81,67a
0,1	42,33b
0,2	22,33c
0,3	19,33cd
0,4	6,67d
0,5	0,00e

Note: The numbers in the same column are followed by a notationthe same letter, showing different values is not realbased on the Duncan Multiple Range Test at the level of 5%

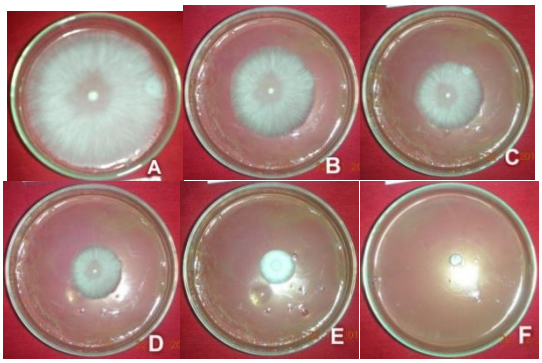

Fig. 5. S.rolfsii colony growth on PDA media treated with jeringau rhizome extract concentration of 0% (A); 0,1% (B); 0,2% (C); 0,3% (D); 0,4% (E); 0,5% (F) after 3 days of incubation

5. CONCLUSIONS

Regarding the research that has been done it can be concluded that the crude extract of the jeringau rhizome has the ability to inhibit the growth of S. rolfsii fungi, with the inhibitory power included in the very strong category and the minimum concentration of crude extract of the jeringau rhizome which is effective for inhibiting S. rolfsii is 0.5 %.

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