Effect of Purified Culture Filtrate of *Rhizoctonia solani* Isolate (UKMRSPL1) on the Detached Fresh Leaf of Parthenium

Siti Nora'rifah Binti Darus

F15A0213

A Thesis Submitted in Fulfilment of the Requirements for the Degree of Bachelor of Applied Science

(Agrotechnology) with Honours

Faculty of Agro Based Industry

Universiti Malaysia Kelantan

2019

DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

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Sign

Student name: Siti Nora'rifah Binti Darus

Matric no: F15A0213

Date:

Approved by:

_____

Sign

Supervisor name:

Cop:

Date:

ACKNOWLEDGEMENT

Alhamdulillah and praise to Allah, the almighty for giving me a healthy life and opportunity to complete this thesis. I would like to express my sincere gratitude to the guidance and supervision of several people. Special appreciation and deepest gratitude to my supervisor, Prof S.M Rezaul Karim and co supervisor Dr Laila Naher for their consistent support of my study degree study and research, suggestion, motivation, patience and valuable from the beginning until the end of the study. Their guidance helped me during the research time and writing of this thesis. This thesis could not be done without their guidance and constant help. I am very grateful to University Malaysia Kelantan for giving me the opportunity to further my study.

Lastly, I would like to express my gratitude to my parent and my family for their financing support and encouragement. I need them to burn my spirit to continue this study without any doubt. Most of all, I am fully indebted to all of them for this understanding, wisdom, patience, and encouragement and also my fellow lab mates Adawiyah and Fatihah who give moral support and encouragement to do the research until the end. Without all of you, I will not be able to do anything.

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LIST OF ABRIVATION SYMBOL

UMKRSPL1	*Rhizoctonia solani* culture from parthenium
R.solani	*Rhizoctonia solani*
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth

LIST OF SYMBOL

%	Percent
&	And
°C	Temperature
mm	Millimetre
g	Gram
ml	Millilitre
L	Litre
rpm	Revolutions per minute

EFFECT OF PURIFIED CULTURE FILTRATE OF *RHIZOCTONIA SOLANI* ISOLATE ON DETACHED FRESH LEAF OF PARTHENIUM

ABSTRACT

Parthenium control by using bioherbicide is highly demanded in Malaysia and elsewhere. A study was conducted to observe the effect of purified culture filtrate of *Rhizoctonia solani* (UMKRSPL1) isolate on the fresh detached leaf of parthenium at the laboratory of University Malaysia Kelantan, Jeli Campus. The isolated was cultured in Potato Dextrose Broth for 10 days. Filtrate was collected and was extracted with methanol and without methanol. The filtrate was purified by treating with activated charcoal. The purified filtrate was centrifuged for 15 minutes and the pallets were collected from the bottom of the centrifuged extracts. The purified and centrifuged extracts were applied on the detached leaf of parthenium at different concentrations of 0%, 2.2%, 5.0% and 10.0%. It was observed that 10.0% extract without methanol caused more 90% killing effect at day 8. The extract without methanol at 10.0% concentration caused only 55% killing effect in comparison to control.

Keywords: Parthenium, *Rhizoctonia solani*, methanol, filtrate, concentration

KESAN PURIFIKASI KULTUR FILTRAT DARIPADA *RHIZOCTONIA SOLANI* ISOLASI DIATAS DAUN PISAHAN SEGAR PARTHENIUM

ABSTRAK

Kawalan parthenium dengan menggunakan bioherbicide sangat mendapat perhatian di Malaysia dan di tempat lain. Satu kajian dijalankan untuk memerhatikan kesan filtrat dari *Rhizoctonia solani* (UMKRSPL1) keatas asingkan daun segar parthenium di makmal Universiti Malaysia Kelantan, Jeli. Isolat berkenaan telah dihidupkan dalam Kentang Dextrose Broth selama 10 hari. Filtrat dikumpulkan dan diekstrak dengan metanol dan tanpa metanol. Filtrat itu ditapis dengan merawat menggunakan arang aktif. Filtrat yang ditapis telah disentrifuged selama 15 minit dan palet dikumpulkan dari bahagian bawah ekstrak. Ekstrak tulen dan sentrifuged digunakan pada daun pisahan segar parthenium pada kepekatan yang berbeza iaitu 0%, 2.2%, 5.0% dan 10.0%. Difahamkan bahawa ekstrak 10.0% tanpa methanol menyebabkan lebih banyak kesan pembunuhan 90% pada hari 8. Ekstrak tanpa metanol pada kepekatan 10.0% disebabkan hanya 55% membunuh kesan berbanding kawalan.

Kata kunci: Parthenium, *Rhizoctonia solani*, metanol, filtrat, kepekatan

CHAPTER 1

INTRODUCTION

1.1 Parthenium invasiveness and mycotoxin extraction

Parthenium weed is an invasive alien species in Malaysia causing problem to human and animal health, and affecting our bio-diversity. This weed has already covered more than 80 hectares of land of 10 different states. Sustainable management of this weed is a national agendum. The weed can be controlled by inorganic herbicides but it leads to environmental pollution.

Development of bioherbicide from natural resources is highly demanded in the country. The UMK scientists, Prof Dr SM Rezaul Karim and Dr Laila Naher have recently isolated a fungus from parthenium leaf coded as UMKRSPL1, which can be used to develop bioherbicide (Karim et al, 2017).

The bioherbicides can be developed by using microbial suspension of the fungus or by isolating the mycotoxins from the culture filtrate of the fungus. The

mycotoxins cause similar kind of disease on the host plant as does by the fungus itself (Singh et al, 2010; Karim et al, 2017).

This study focuses on the development of bioherbicide by extracting mycotoxins of the fungus. Therefore, it is important to know the appropriate concentration of mycotoxin containing extract to get maximum killing effect on the parthenium leaf.

1.2 Problem statement

Parthenium hysterophorus L. has been spreading widely in Malaysia, covering 10 states including Sabah (Karim, 2017). Although the weed can be controlled by using chemical herbicide, it leads to environmental pollution. Biological control using mycotoxin derived from parthenium fungi is an eco-friendly and sustainable method of control. No information especially on the bio control of parthenium weed using bio-herbicides is available in Malaysia.

1.3 Objective

I. To study the effect of methanol-extracted filtrate (containing mycotoxins) on the disease infection of parthenium leaf.

II. To determine the appropriate concentrations of mycotoxin filtrate to create deadly disease infection on parthenium leaf.

1.4 Limitation of the study

Possibility of contamination with other fungus during laboratory cultivation. Requirement of permission to from Department of Agriculture to bring the fresh leaf of parthenium to the UMK laboratory at Jeli. (Likely, we have obtained the permission).

1.5 Scope of study

This study is related to develop of biocontrol method of parthenium weed, and thereby to sustain our environment.

1.6 Hypotheses

1. Culture filtrate of UMKRSPL1contain mycotoxin which cause disease to parthenium leaf.
2. Purified culture filtrate is effective to create disease on parthenium leaf.
3. The action of culture filtrate on the freah leaf of parthenium can be varies under different concentrations of filtrate.

CHAPTER 2

LITERATURE REVIEW

2.1 Impact of parthenium weed on agriculture and environment

Parthenium hysterophorus L. is a weed that can affect the ecosystem in a country. The weed has infected agricultural project in the country that inhibit the growth of the parthenium weed. The example of country that is affected by parthenium weeds are India, Pakistan, Bangladesh, Nepal, Sri Lanka, Australia, Malaysia and Ethiopia. The effect of the weed on the ecosystem includes habitat changes in the grasslands, river bank, woodland and food plains (Chembolli & Srinivas, 2007).

Parthenium weed contain chemical such as parthenin, hysterin, hymenin and ambrosin, that will exerts strong allelopathic effect on different plant (Gunaseelan, 1998). The weed can affect the nodulation in legumes due to activity of inhibiting and nitrifying the bacteria (Kaur & Aggarwal, 2017). In Bangladesh, a survey shows

that the parthenium weed can grow and inhibit in different land type, such as road side, homestead, low land and railway track (Akter & Zuberi, 2009).

A study was conducted on the effect of parthenium on maize plant. The result showed that the maize plant could not withstand the existence of Parthenium weed in the land. Parthenium weed can reduce the grain yield, biological yield, grain weight per cob and the harvest index of maize (Safdar, Asif & Rias, 2015).

A study was conducted in the grazing land in northeastern Ethiopia. The existence of Parthenium weed in the land has caused a serious threat to the farming economy in Ethiopia. The Parthenium destroyed the grazing land and affected the farming activity in that area (Lisanework, Asresie, Janmejai, & Steve, 2010).

Parthenium hysterophorus L. has also affected Islamabad, Pakistan. Parthenium weed affected the livestock, crop production and biodiversity of Islamabad. The aggressive colonization of parthenium weed also caused the reducing of the common medicinal plants in the wasteland of Islamabad (Asad, & Rukhsana, 2006).

The spreading of seed of Parthenium weed can cause potential seed contamination on the grain and other crop. It cause double cultivation cost and cause the restriction of the process of exporting the grain and crops. The growth of Parthenium weed in a landscape, nursery or ornamental crop, cause the competition between ornamental plant with the weed. Some of the ornamental plant may die and this may increase the weed management cost.

Parthenium hysterophorus L. can cause disease to the crops and animals. It shows that cattle and horses can be infected with dermatitis with skin lesion

(Jayaramiah et al, 2017). Dermatitis can infect the skin and around the bulb of the heels (Laven, 2001). Furthermore, the weed can cause anorexia, diarrhea and eye irritation on dogs. It can also cause bitter milk and tainted milk for buffalo, cow and goats (Aneja, 1991).

Parthenium weed can be one of the reasons for the affecting human health and environment. Farmers that dealing with Parthenium weed suffer with allergic reaction, asthmatic problem, stomach pain, stretching and cracking of skin and contraction of breath muscle (Karim et al, 2013).

2.2 Uses of parthenium weed

Parthenium has been recognized as the potential source of bioherbicide (Karim et al, 2017). Bioherbicide is the material containing a biological agent to control of some weeds. Parthenium weed tested onto 3 type of weeds, *Triticum aestivum, Avena fatua* and *Lepidium sp.* They observed that the extract of Parthenium weed help in reducing the germination, seedling length and seedling weight of some weeds (Marwat et al, 2008).

In several studies, it is found that Parthenium weed can be used as compost and biocontrol agents. Use of Parthenium weed in agriculture can help in reducing the spreading of the crop weed (Prem, Ghosh, Surendra, & Maurya, 2010).

A present study shows that the green manure from the Parthenium weed can be used as an alternative of NPK fertilizer. Parthenium can help in improving the yield and growth of the wheat (Arshad & Shah, 2010).

A study conducted by shows that, the compost from parthenium weed contain higher N, P, and K content than farm yard manure (Bhoyar et al, 2014). Parthenium weed used in biogas production, where it is added with cattle manure to undergo anaerobic fermentation at room temperature (Gunaseelan, 1987). In controlling insect, the parthenium weed extract is used to control mustard aphid on *Brassica juncea* (Sohal et al, 2002). In the industry, the parthenium weed can transform the herbicide, insecticide, cardboard and needed in removing basic dye (Bhoyar et al, 2014).

2.3 Toxin in weeds

Toxin is defined as a poisonous substance that is a specific product of the metabolic activities of a living organism and is usually very unstable, notably toxic when introduced into the tissues, and typically capable of inducing antibody formation. The major component of toxin of parthenium is parthenin and other phenolic acids such as caffeic acid, vanillic acid, anisic acid, panisic acid, chlorogenic acid and parahydroxy benzoic acid (Satao & Shinde, 2014). Plant toxin can be found naturally on the food, weeds, feed and ornamental plants. Plant toxin is

a secondary metabolites that is produced by the plants itself to protect the plants from threats such as bacteria, fungi and insects (Satish et al, 2013).

Toxin can bring useful and harmful to human being and animals. It can cause thyroid, skin problem and neurological syndrome. Plants toxin can diffuse and enter into body by inhalation, swallowing and in contact with the plants. Children are infected by the toxin faster and effectively than adults. The toxin infection depends on the protein, steroids and others respond towards it (Satish et al, 2013).

Plant toxin can be found on the leaf, flower, pollen, seed, root, fruit, stalk and other parts. The difference between the high and low in the toxin infection is due to the infection of the plants.

Several species are known to be toxic when consumed by animals. *Parthenium hysterophorus* L. were found to cause chronic or acute toxicity to animals when being consumed in dietary livestock (Narasimhan et al, 1980). A purified toxin from the parthenium sample were identified as sesquiterpene lactone parthenin, according to the comparison with the properties of sample (Narasimhan et al, 1980).

Earlier study shows that the sesquiterpene lactone parthenin had contribute to the toxicity to the livestock (Narasimhan et al, 1984). The major components of the toxin is parthenin and phenolic acid such as ansic acid, chlorogenic acid, parahydroxy benzoic acid and caffeic acid (Pankaj, 2001). Parthenium found to cause effect on human and livestock such as asthma, bronchitis, dermatitis and hay fever.

2.4 Mycotoxins in weed plants

The presence of mycotoxins can cause illness and death in livestock, and pose potential hazard to human health. Mycotoxins cause disturbance in economic by causing loss in crop production (Herman, 2000). The common mycotoxins are aflatoxins, ochratoxin A, fumonisins, deoxynivalenol, T-2 toxin and zeatalenone (Muneera Al-Kahtani, 2014).

Mycotoxins is found to be available in the wheat flour production. The mycotoxins enters during the food chain process in the field and continue attacking the products during shipping, handling and storing process (Bennett & Klich, 2003). Study shows that mycotoxins is hazardous than plant toxins, food additives, or pesticide residue (Kuiper-Goodman, 1998).

A practice to control the mycotoxins is to control the disperse of mycotoxins disease to other plants. Some method is introduced such as avoiding water stress, minimize insect infestation, reduce inoculum potential, use appropriate drying technique, keep in proper storage and keep out from moisture to prevent moulds growth (Adeyeye, 2016). Mycoherbicides is defined as plant pathogenic fungi that can help in controlling weeds in the way of chemical herbicides (Tebeest & Templeton, 1985: El-Sayed, 2005).

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Materials required

a) Stock culture of UMKRSPL1.

b) Potato dextrose agar (PDA) powder.

c) Potato dextrose broth (PDB) powder.

d) Methanol.

e) Distilled water.

f) Petri dish.

g) Autoclave.

h) Conical flask.

i) Centrifuge.

j) Filter paper.

k) Activated charcoal.

l) Shaker.

3.2 Methodology followed

3.2.1 Cultivation of fungal culture in PDA and PDB media

I. Preparation of PDA media:

a. The Potato Dextrose Agar (PDA) was used to culture the UMKRSPL1 isolate. 19.5g of commercial PDA powder was placed in a conical flask containing 500 ml of distilled water. The solution was mixed together and was homogenated. After that, the media was placed in the media bottle to be autoclave for 15 min at 121°C. After autoclaving process, the media was added with 5 ml of streptomycin. Then, about 9 ml of PDA media was poured into the Petri dish.

II. The culturing of fungi in PDA

b. Culture plate preparation

In the culturing of fungi in Potato Dextrose Agar, the first method is by the transferred loop was sterilized by holding the wire in the flame until it is red hot. The loop was lets cool. Then, the cap was removed while holding the sterile loop and culture and the mouth of the plate was heated in the flame before inserting needle or loop. Later, the agar block containing fungal structures was transferred from the culture and was transferred onto fresh PDA agar. The mouth of plate was heated and

close. The Petri dish containing the pieces was sealed using Parafilm. Lastly, the petri dish was incubated at temperature of 25°C+2 until growth has appeared.

III. The culturing of the fungal isolate in potato dextrose broth

The first method is the potato dextrose broth was made by adding 36 g of potato dextrose broth into 1.5 L of distilled water followed with the broth was autoclaved for 15 minutes. Then, the potato dextrose broth was transferred into 100 ml of conical flask. After the broth is cool down, UMKRSPL1 fungal isolate was added to the conical flask by using cock borer and each conical flask was provided with 4 circles the culture and was incubated for ten days. After that, the culture filtrate was obtained by filtering the metabolized growth medium through pre-weighed Whatman filter paper No1. Lastly, the crude extract was filtered through filter paper.

3.2.2 Extraction of UMKRSPL1 culture with methanol

After complete growing of the UMKRSPL1 in potato dextrose broth, the fungal mass was separated by filtration with Whattman filter paper (Figure 3.1).

Then, the content was treated with 80% methanol solution (Al-Kathani, 2014).

The filtrate was in light yellow in color and in clear solution (Figure 3.2).

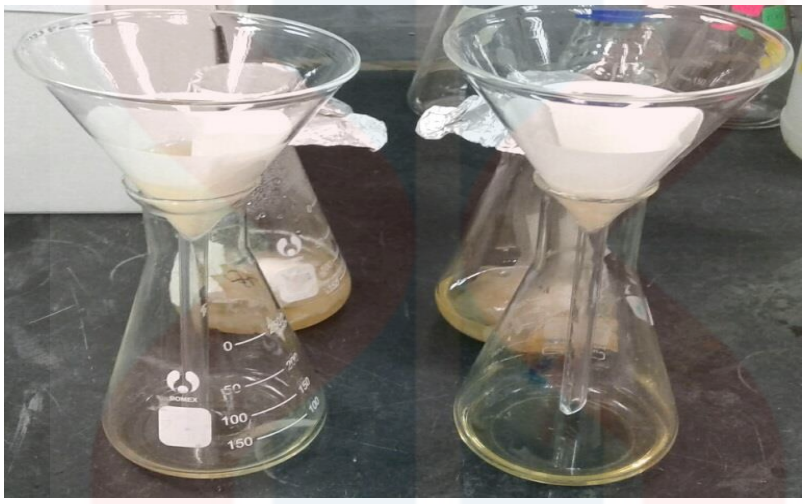


Figure 3.1 The filtering process of UMKRSPL1 culture with Whattman filter paper No. 1

Figure 3.2 The filtrate of culture

The purpose of using methanol is to extract the mycotoxins present in the culture filtrate. Mycotoxins may be soluble in different solvent. A study shows that methanol results in highest extraction efficiency than other extract solution (Wang et al, 2013).

An amount of 400 ml of culture filtrate was mixed with 100 ml of mixture of methanol and distilled water at ratio of 80 : 20 and was shaked at 200 rpm for 1 hour at 25°C. The extract was filtered by using Whattman No. 1 filter paper. Then, the filtrate solution was shaked for one hour at 200 rpm. The process of shaking the solution would help in isolation of mycotoxin (Figure 3.3).

Figure 3.3 The solution extracted with methanol was shaked

3.2.3 Purification by Activated charcoal

After extracting the filtrate with methanol, the solution with methanol and without methanol was purified by using activated charcoal (Figures 3.4-3.7). The activated charcoal acts as universal absorbent because the activated charcoal can binds itself with variety molecules (Chandy & Sharma, 1998). 400 ml of culture filtrate was treated with 5 gram of activated charcoal and was filtered by using Whattman No.1 filter paper. The filtrate obtained from charcoal treatment was evaporate to dryness in vacuo at 50°C.

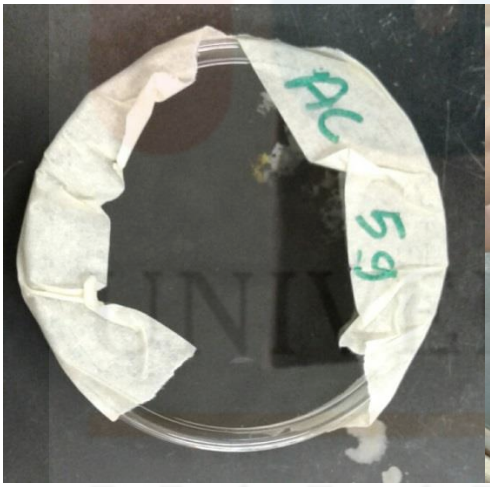


Figure 3.4 The activated charcoal

Figure 3.5 The solution added with activated charcoal

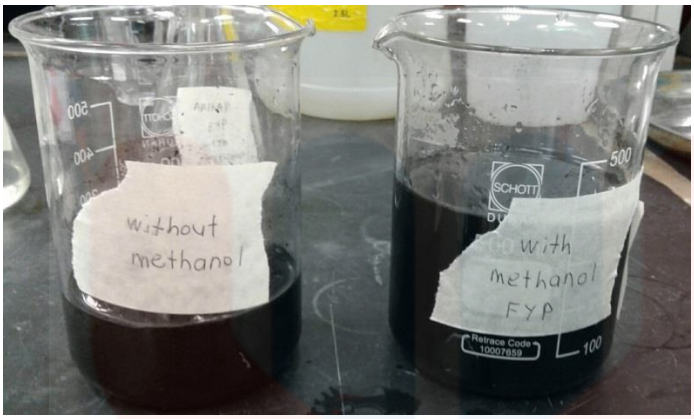


Figure 3.6 The purification of solution with activated charcoal.

Figure 3.7 Filtration of charcoal-treated solution with filter paper

Centrifusion process were done for fifteen minutes at 10,000 rpm after filtering the solution mixed with activated charcoal. This process is important to get the pallet and supernatant from the filtrate solution. The pallet obtained were evaporate to dryness in vacuo to get the thicker concentration of solution (Vidhyasekaran et al, 1997).

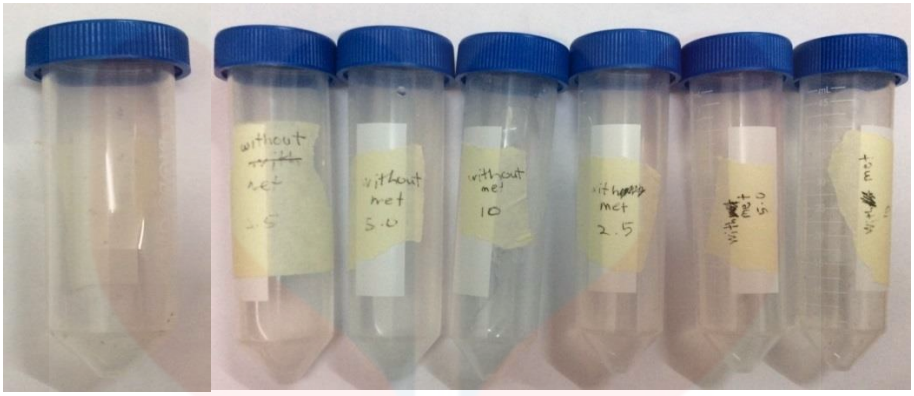


Figure 3.8 The control and the treatment solutions after centrifuge process

3.2.4 Collection of parthenium leaf

The parthenium leaf was collected from infested area at Kedah. The infested area were located nearby the paddy field and a housing areas (Figures 3.9).

Figure 3.9 The place of sample collection at Kedah

3.2.5 Pathogenicity test of partly purified filtrate on the fresh leaf of parthenium:

Pathogenicity test was done to know the killing effects of the culture filtrate (McGrath, 2005), following the below-mentioned procedure.

Firstly, the fresh and and young sample was choosed from the infected area and carried to UMK laboratory using cool box. The sample was sterilized by using sodium hypochloride (0.01%) for 10 seconds and the sample was wiped using clean tissue, followed with the sample was put on the cotton pad. Once the sample leaves was put in a sterile petri dish, it the sample was dropped with partly purified filtrate at the rate of 10 drops per leaf and was incubated in dark place for 10 days at room temperature. Lastly, the disease symptom developed on the leaves was evaluated by scoring system (Dey, 2010).

Grade Description:

0 = No symptom on in any part of the leaf.

1 = Minute brown lesion on the leaf.

2 = Moderately brown lesion on the leaf.

3 = Leaf symptom discolored and yellowed.

4 = More than 50% of leaf are yellowed.

5 = Severe yellowing all over the leaf.

6 = Production of necrotic leaf or dying of leaf.

Figure 3.10 The leaf was cleaned by using distilled water

The cleaned parthenium leaf was placed in a petri dish and the extract was added at different concentrations as per treatment specification (Figure 3.11).

Figure 3.11 The parthenium leaf was soak in sodium hypochloride for 10 seconds

3.3 Data collection

Percentage of damage or disease symptoms occurred on parthenium leaf due to application of different solutions was evaluated by scoring method (Dey, 2010). The percentage of the damage or disease symptom occurred on parthenium leaf was obtained from the Appendix I. Appendix I Raw data showing the effect of cell filtrate of UMKRSPL1 on the disease infection on detached parthenium leaf (disease scores).

3.3.1 Disease Scoring

The effectiveness of culture filtrate of UMKRSPL1 as mycoherbicide was determined based on the disease symptoms grade (Dey, 2010). The disease symptom was evaluated from the picture of the effect of treatment on the detached Parthenium leaf (refer Appendix II).

Grade Description:

0 = No symptom on in any part of the leaf.

1 = Minute brown lesion on the leaf.

2 = Moderately brown lesion on the leaf.

3 = Leaf symptom discolored and yellowed.

4 = More than 50% of leaf are yellowed.

5 = Severe yellowing all over the leaf.

6 = Production of necrotic leaf or dying of leaf.

(Modified from: Dey, 2010)

3.4 Statistical analysis

The data on percentage of damage on parthenium leave was analysed following the technique of ANOVA analysis.

CHAPTER 4

RESULT AND DISCUSSION

The whole study was completed under two major headings as below:

A. Reconfirmation of *Rhizoctonia solani* isolate through morphological and microscopic study.
B. Quantification of killing effect of purified culture filtrate on the fresh detached leaf of parthenium.

The findings of the study are given below under those headings:

4.1 Reconfirmation of *Rhizoctonia solani* isolate (UMKRSPL1) through morphological and microscopic study.

4.1.1 Macro morphological identification of *Rhizoctonia solani* fungus from UMKRSPL1 culture.

The development of sclerotia and mycelia development within the potato dextrose agar and grown on the surface of the fungul culture are confirmed by the presence of dark spot-like sclerotia.

Figure 4.1 The morphology of UMKRSPL1 culture on potato dextrose agar

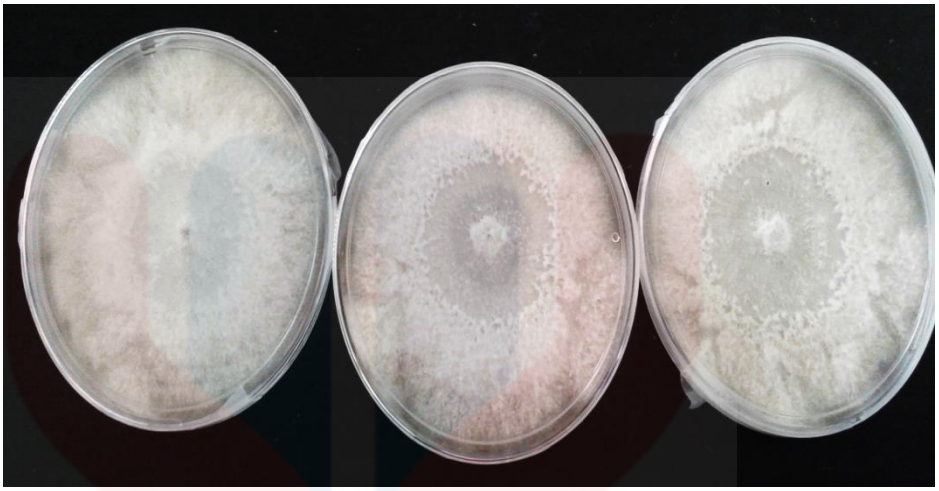


Figure 4.2 The morphology of UMKRSPL1 culture on potato dextrose agar (blackish ring of sclerotia in centre)

As observed from the figures above, the culture grows into a dense white mycelia with black dot on the potato dextrose agar. Generally the young mycelia of *R. solani* initially produce white mycelia that will turns into brown blackish in color. Finally, it turns to blackish in color. The sclerotia developed are in different shapes and sizes. Temperature and nutrient might affect the size and shape of the sclerotia (Thiessen & Woodward, 2012).

4.1.2 Microscopic identification

The views of the isolated fungus under stereomicroscope has been given in Figures 4.3 until 4.5. After observing under stereomicroscope, it shows that a huge mass of mycelia was observed on the potato dextrose agar culture. A wet mount slide technique was used before observing the slide under stereomicroscope. A small portion of the culture was picked and scooped out by using a sterilized loop and placed on the slide and then covered with cover slip.

R. solani is composed of thin, vegetative strands called as hyphae. The hyphae can be recognized by the right and acute angles to the main hyphae. It is observed that the hyphae produced at 90° and 45° angle of branch rise from the main hyphae. The hyphae was composed of compact cells known as moniloid, that fuse together later and form hard structure called as sclerotia (Tredway & Burpee, 2001).

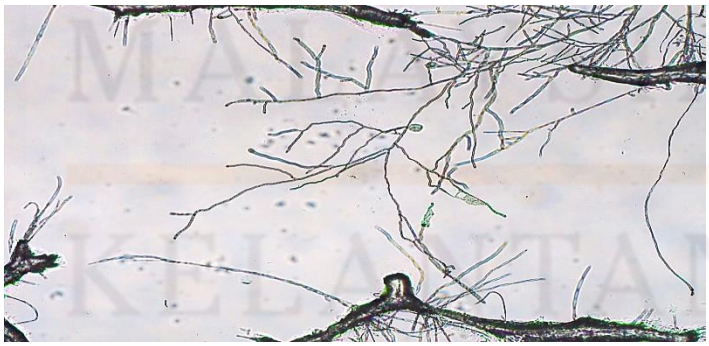


Figure 4.3 The fungal structure under 10x magnification

The characteristic of the multi-nucleous hyphal cells was observed under stereomicroscope and the morphological characters of the species was matched with the key characteristic as described by others. Based on the observation, the branch was found to be at the right and acute angle of the hyphae. Sclerotia was found on the potato dextrose agar. As observed from the figures, the septum from the main hyphae was thicker and larger in size than the secondary septum (Figures 4.4 & 4.5).

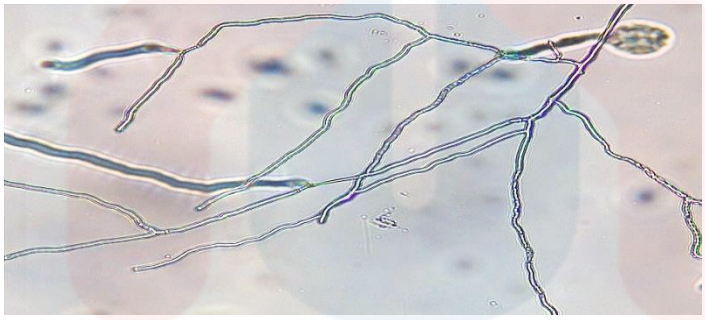


Figure 4.4 The fungal under 10x magnification

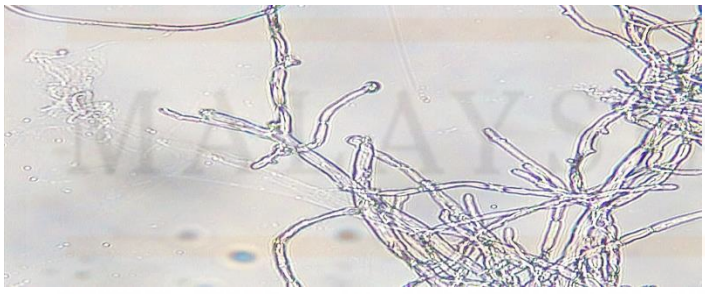


Figure 4.5 The fungal under 40x magnification

4.1.3 UMKRSPL1 culture in potato dextrose broth

The UMKRSPL1 fungal isolate from potato dextrose agar was cultured in potato dextrose broth for 14 days at 26°C. Four circular 6 mm size of culture were grown in 100 ml of potato dextrose broth until it produced a sufficient growth of the UMKRSPL1 culture (Figures 4.6-4.9).

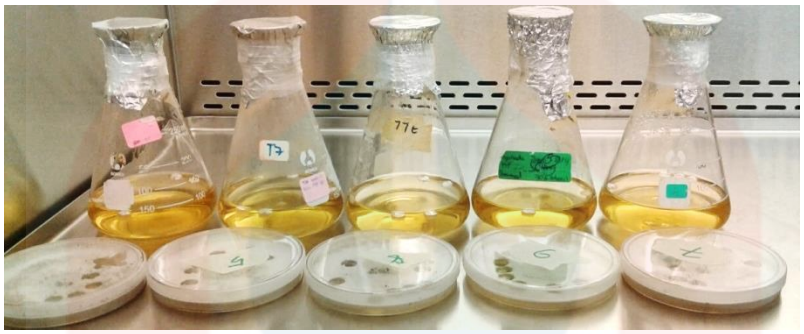


Figure 4.6 The UMKRSPL1 culture and potato dextrose broth

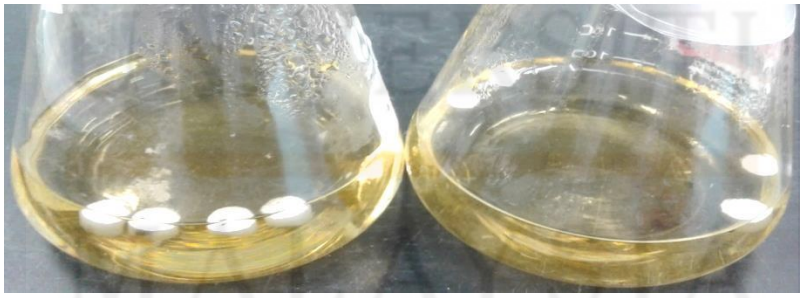


Figure 4.7 Four circular 6 mm size UMKRSPL1 culture in potato dextrose broth.

Figure 4.8 UMKRSPL1 culture on potato dextrose broth on day 3

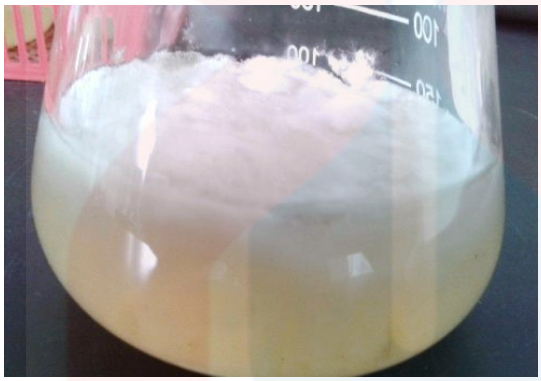


Figure 4.9 The fully grown UMKRSPL1 culture in potato dextrose broth

After incubating for 7 days the UMKRSPL1 culture in potato dextrose broth was increased in size. Finally, increased until covering the entire surface area of 100 ml of potato dextrose broth. The potato dextrose broth supported the abundant growth of the UMKRSPL1 isolate because potato dextrose broth is the most nutritive media for *R. solani* (Khan, 2016).

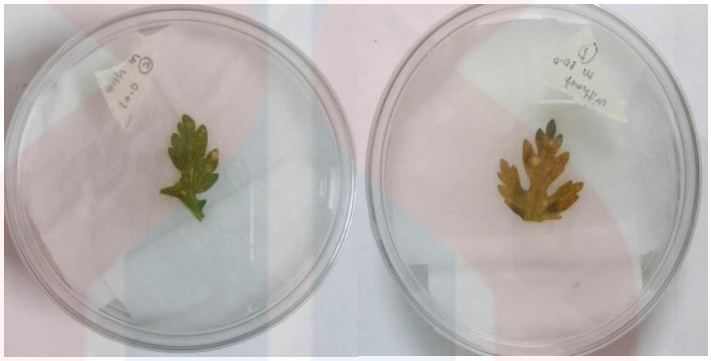
4.2 Effect of purified culture filtrate on detached parthenium leaf

The purified culture filtrate has produced very clear killing effect on the parthenium leaf (Table 1.0, Appendix III). The effect increased with the increase of treatment duration (days). However, within 8 days of treatment more than 90% killing effect was observed (Appendix II & III).

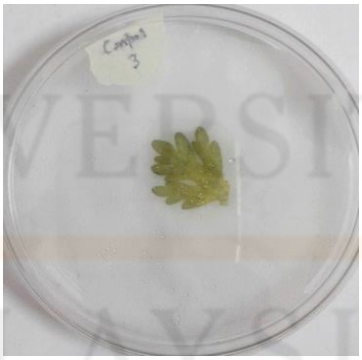
Table 1.0 Effect of different concentrations of fungal cell filtrates and time of incubation (day) on the disease development on detached parthenium leaf (figures are mean of disease scores)

Treatment	Time of incubation						Mean
	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	
Control (0%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
-Metha 2.5%	0.00	1.00	2.00	2.67	4.33	5.00	2.50
-Metha 5.0%	0.00	1.67	3.00	3.33	5.00	5.00	3.00
-Metha 10.0%	0.00	2.67	3.33	4.00	5.67	5.67	3.56
+Metha 2.5%	0.00	0.67	1.00	2.67	3.67	4.00	2.00
+Metha 5.0%	0.00	0.33	1.00	1.67	3.67	5.00	1.94
+Metha 10.0%	0.00	1.00	1.67	2.67	2.67	3.00	1.83

From the below-mentioned photographs in Figure 4.10 it is obvious that on the day 8, the parthenium with control treatment is still green in color but the leaf with the treatment of 10% extract without methanol caused almost100% yellowing of the leaf. The treatment with 10% concentration and with methanol caused about 55% yellowing.



A B



C

Figures 4.10 Effect of extract of culture filtrate with (A) and (B) without methanol along (C) with the control (without extract) treatment

Although in some cases, further increase has been noticed in day 10, the rate of increase was low (Figures 4.11).

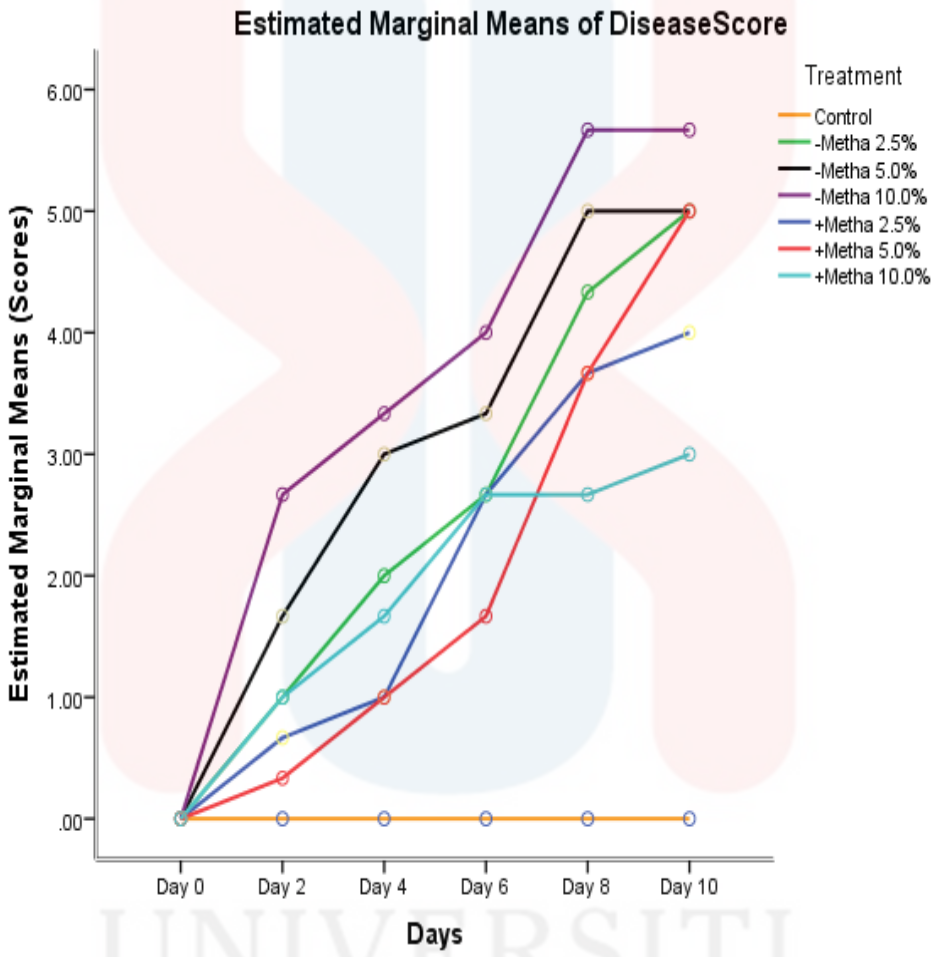
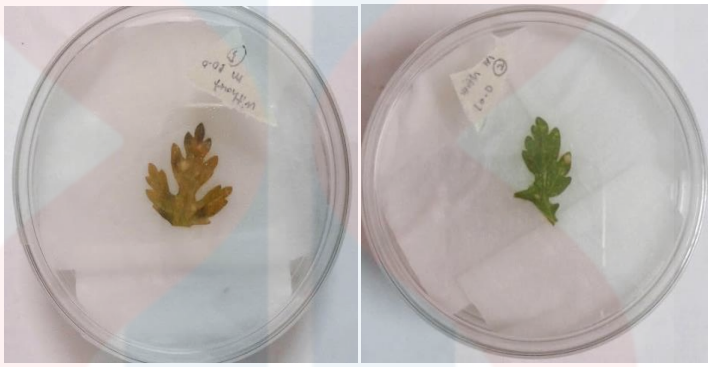


Figure 4.11 Effect of different treatments of the extract on the disease score of parthenium leaf over time

Among the treatment, the extract without methanol produced good killing effect than the extract with methanol (Figures 4.11). On an average more than 36% killing effect has been noticed in case of extract without methanol

(Table 1.0). The effect was concentration dependent. The highest effect (more than 90%) was found to 10% extract without methanol. Under some concentration, the extract with methanol caused 53% less effect than without methanol (Figures 4.12).



A B

Figures 4.12 Effect of 10% extract without methanol (A) and with methanol (B) on day 8

CHAPTER 5

CONCLUSION

The purified cell filtrate of the UMKRSPL1 fungal isolate is effective to create disease symptoms on the parthenium leaf, which indicates that it is a good candidate for bioherbicide development for parthenium control.

The culture filtrate without methanol is better than with methanol to create the killing effect on the parthenium leaf. As purified cell filtrate at 10% concentration is sufficient to get more than 90% killing effect of the parthenium leaf.

Eight days after treatment is enough to produce more than 90% killing effect of parthenium leaf.

RECOMMENDATION

Further studies should be done to see the killing effect of the cell filtrate on attached parthenium leaf under field condition. Identification of mycotoxin, which are responsible for killing effects should be identified through biochemical analyses. More study through biochemical should also be carried out to know how to enhance the extraction of mycotoxins in Potato Dextrose Broth.

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APPENDICES

Appendix I Raw data showing the effect of cell filtrate of UMKRSPL1 on the disease infection on detached parthenium leaf (disease scores).

Treatment	R1	R2	R3
Day 0			
Control (0%)	0	0	0
-Metha 2.5%	0	0	0
-Metha 5.0%	0	0	0
-Metha 10.0%	0	0	0
+Metha 2.5%	0	0	0
+Metha 5.0%	0	0	0
+Metha 10.0%	0	0	0
Day 2			
Control (0%)	0	0	0
-Metha 2.5%	2	0	1
-Metha 5.0%	2	2	1
-Metha 10.0%	2	3	3
+Metha 2.5%	0	1	1

+Metha 5.0%	0	1	0
+Metha 10.0%	1	1	1
Day 4			
Control (0%)	0	0	0
-Metha 2.5%	2	2	2
-Metha 5.0%	3	3	3
-Metha 10.0%	3	3	4
+Metha 2.5%	1	1	1
+Metha 5.0%	1	1	1
+Metha 10.0%	1	2	2

Treatment	R1	R2	R3
Day 6			
Control (0%)	0	0	0
-Metha 2.5%	3	2	3
-Metha 5.0%	3	4	3
-Metha 10.0%	4	4	4
+Metha 2.5%	3	3	2
+Metha 5.0%	2	2	1
+Metha 10.0%	2	3	3
Day 8			

Control (0%)	0	0	0
-Metha 2.5%	4	4	5
-Metha 5.0%	5	5	5
-Metha 10.0%	5	6	6
+Metha 2.5%	4	4	3
+Metha 5.0%	4	4	3
+Metha 10.0%	2	3	3
Day 10			
Control (0%)	0	0	0
-Metha 2.5%	5	5	5
-Metha 5.0%	5	5	5
-Metha 10.0%	5	6	6
+Metha 2.5%	3	4	5
+Metha 5.0%	5	5	5
+Metha 10.0%	3	3	3

Appendix II Pathogenicity test on parthenium leaf with different treatments of extracts of culture filtrates during different periods of time.

Day 0

1. Control with distilled water

2. Without methanol 2.5 %

3. Without methanol 5.0 %

4. Without methanol 10.0 %

5. With methanol 2.5 %

6. With methanol 5.0 %

7. With methanol 10.0 %

Day 2

1. Control with distilled water

2. Without methanol 2.5%

3. Without methanol 5.0%

4. Without methanol 10.0%

5. With methanol 2.5%

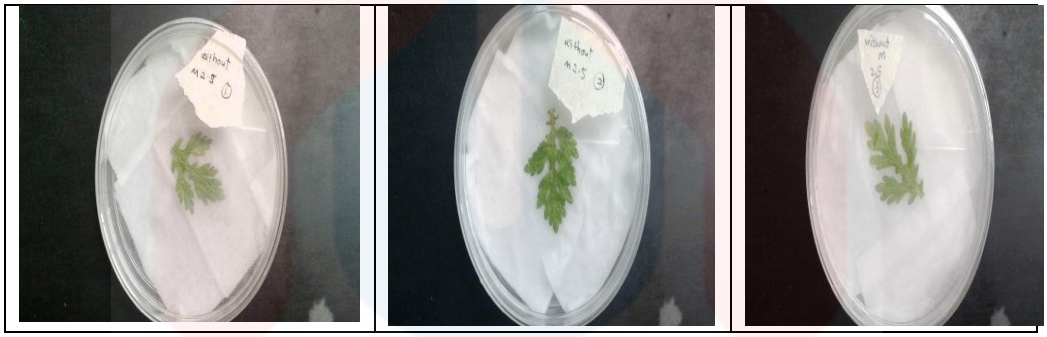
6. With methanol 5.0%

7. With methanol 10.0%

Day 4

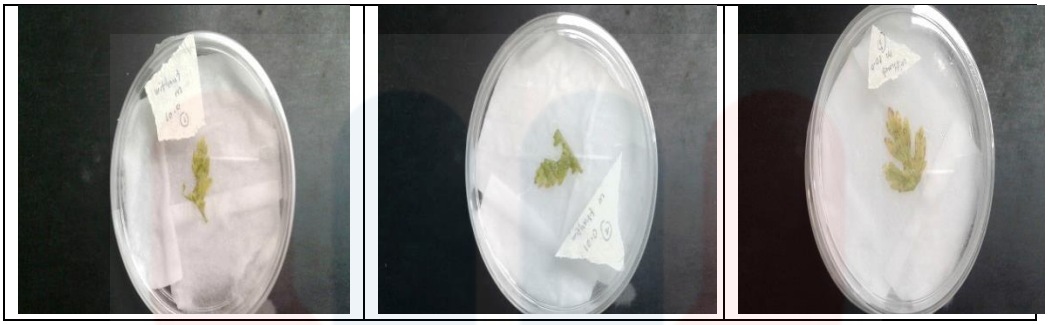
1. Control with distilled water

2. Without methanol 2.5%



3. Without methanol 5.0%

4. Without methanol 10.0%



5. With methanol 2.5%

6. With methanol 5.0%

7. With methanol 10.0%

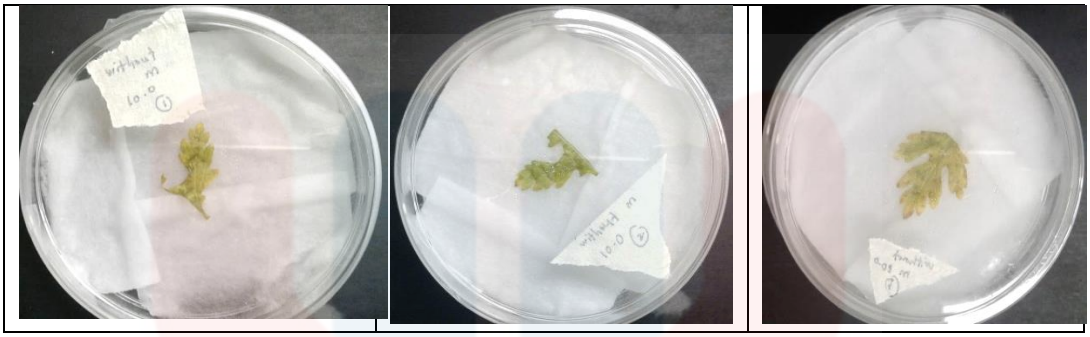
Day 6

1. Control with distilled water

2. Without methanol 2.5%

3. Without methanol 5.0%

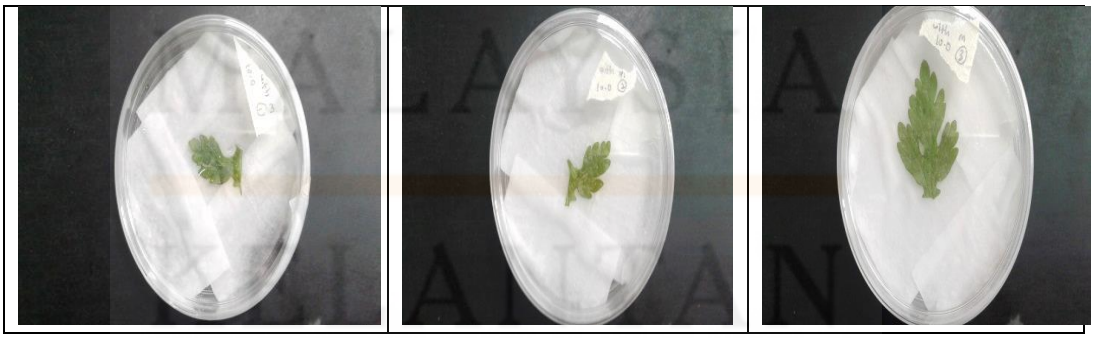
4. Without methanol 10.0%



5. With methanol 2.5%

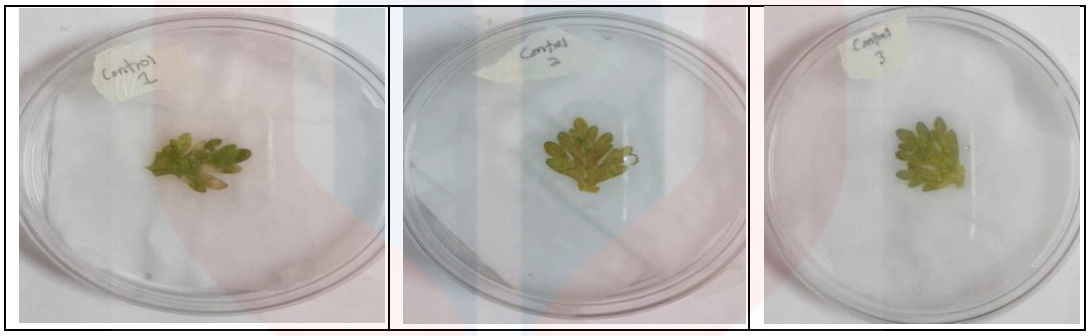
6. With methanol 5.0%

7. With methanol 10.0%

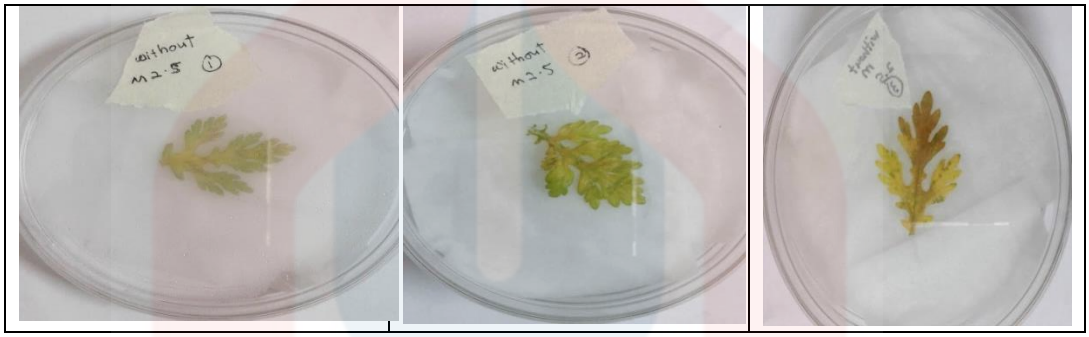


Day 8

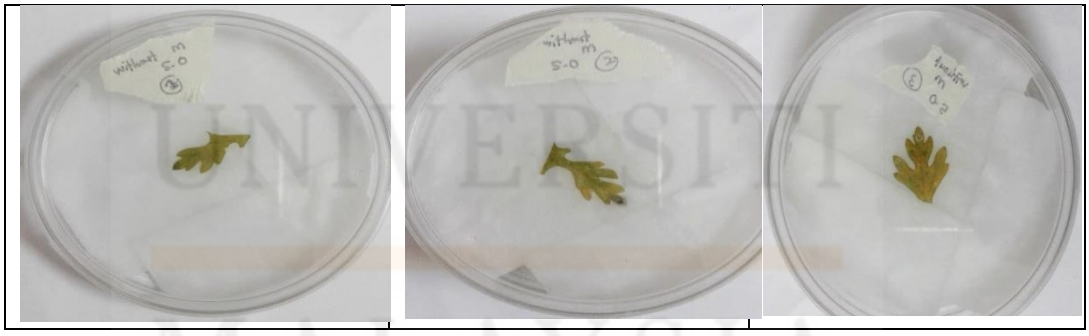
1. Control with distilled water



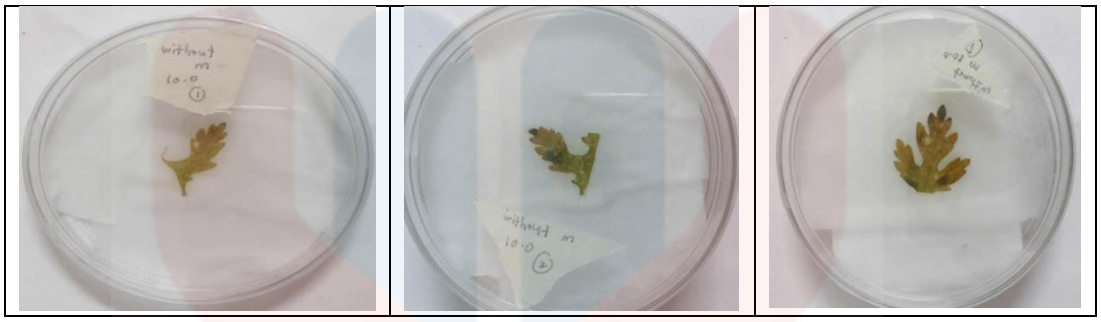
2. Without methanol 2.5%



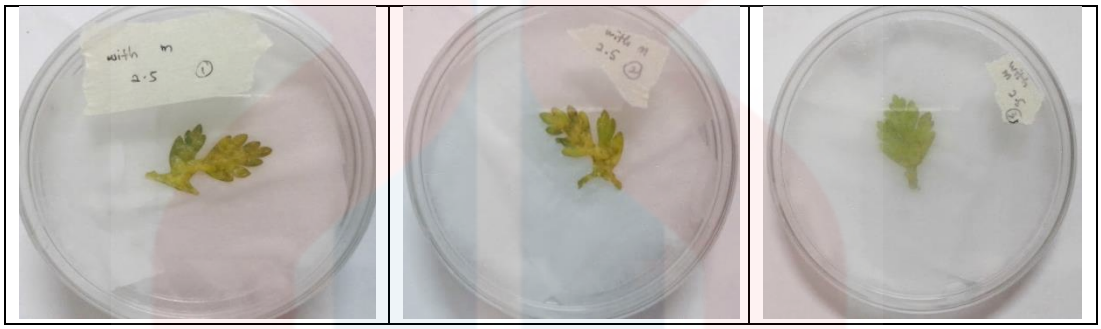
3. Without methanol 5.0%



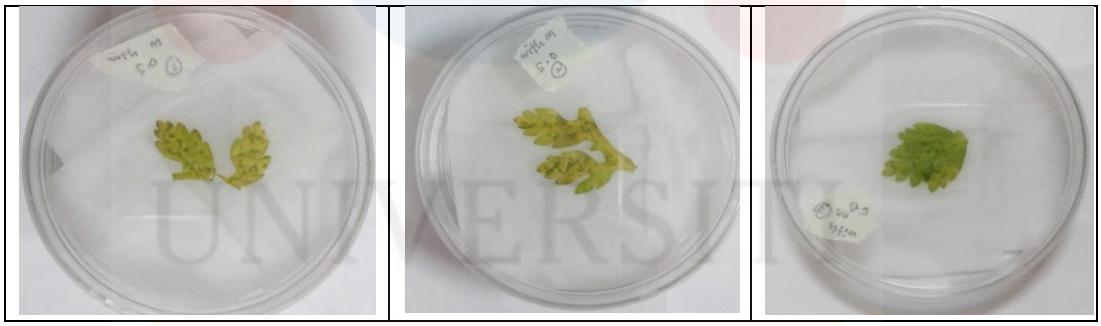
4. Without methanol 10.0%



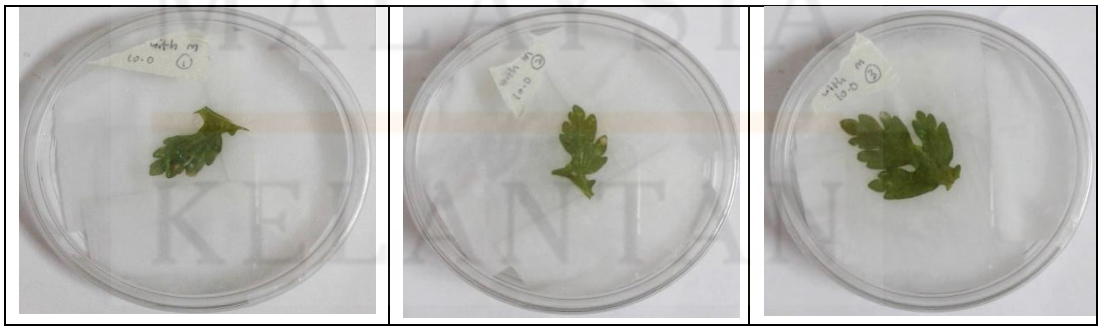
5. With methanol 2.5%



6. With methanol 5.0%



7. With methanol 10.0%



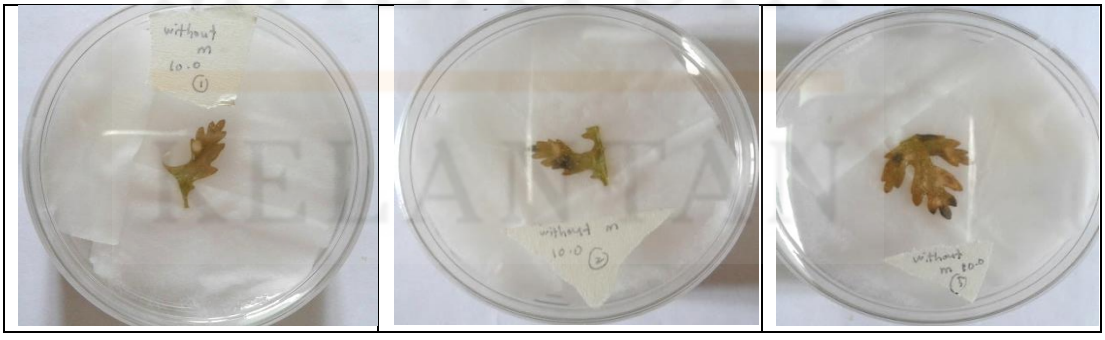
Day 10

1. Control with distilled water

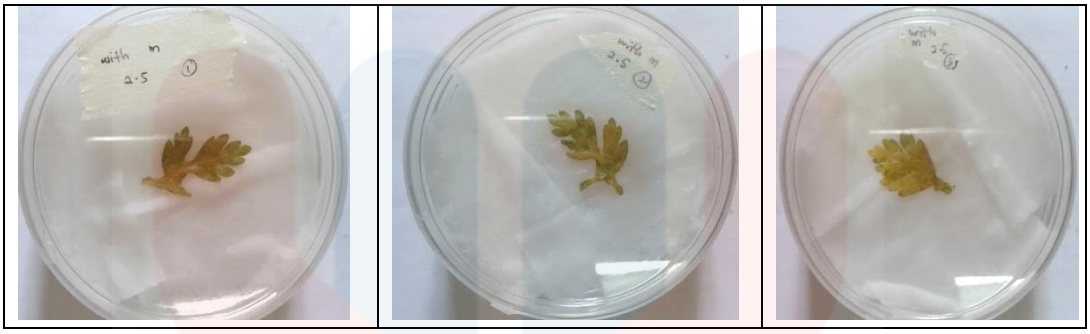
2. Without methanol 2.5%

3. Without methanol 5.0%

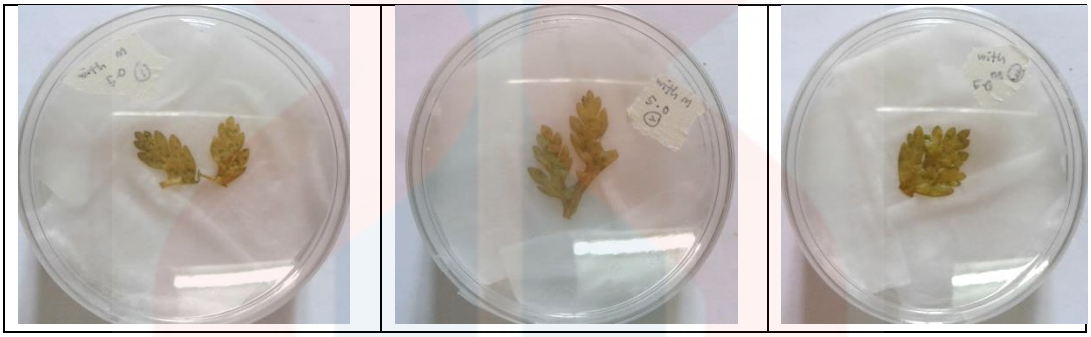
4. Without methanol 10.0%



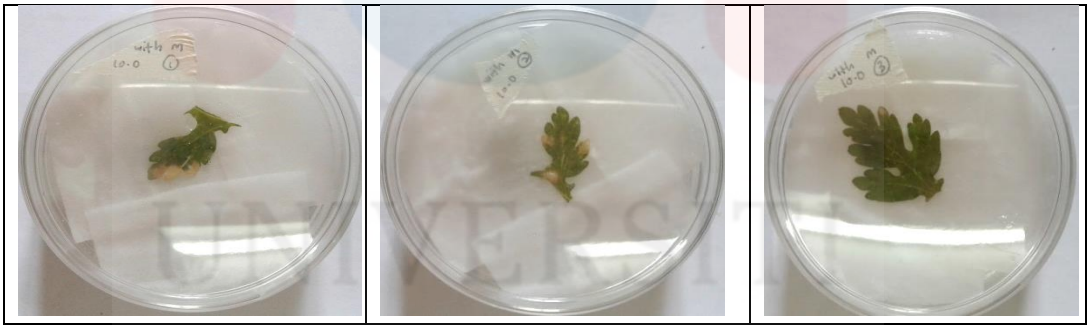
5. With methanol 2.5%



6. With methanol 5.0%



7. With methanol 10.0%



Appendix III ANOVA table showing effect of different treatment and days of incubation on disease scores on parthenium leaf.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	437.881[a]	41	10.680	58.508	.000
Intercept	565.786	1	565.786	3099.522	.000
Day	238.738	5	47.748	261.574	.000
Treatment	136.825	6	22.804	124.928	.000
Day * Treatment	62.317	30	2.077	11.380	.000
Error	15.333	84	.183		
Total	1019.000	126			
Corrected Total	453.214	125			