

# Assessment and evaluation of the potential of fungi agents against Parthenium ( *Parthenium hysterophorus* L.) Weed in relation to chemical herbicide



## Original Research Article

ISSN : 2456-1045 (Online)  
 (ICV-AGS/Impact Value): 63.78  
 (GIF) Impact Factor: 4.126  
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 Journal Code: ARJMD/AGS/V-29.0/I-1/C-II/SEP-2018  
 Category : AGRICULTURAL SCIENCE  
 Volume : 29.0 / Chapter- II / Issue -1(SEPTEMBER-2018)  
 Journal Website: [www.journalresearchijf.com](http://www.journalresearchijf.com)  
 Paper Received: 27.07.2018  
 Paper Accepted: 22.08.2018  
 Date of Publication: 05-10-2018  
 Page: 05-10



Name of the Author (s):

Samuel Sahile<sup>1</sup>,  
 Nigest Asmelash<sup>2</sup>

<sup>1,2</sup> College of Natural and Computational Science, University of Gondar, P.O.Box 309, Gondar, Ethiopia.

## Citation of the Article

Sahile S ; Asmelash N. (2018) Assessment and evaluation of the potential of fungi agents against Parthenium ( *Parthenium hysterophorus* L.) Weed in relation to chemical herbicide.; *Advance Research Journal of Multidisciplinary Discoveries*.29(2)pp. 05-10

## ABSTRACT

**P**arthenium (*Parthenium hysterophorus* L.) weed is an annual herb in the family Asteraceae which is characterized by deep tap root, pale green leaves and an erect stem that becomes woody gradually. It is very harmful to human, animal, agriculture and environment. The aim of the present study is to assess and evaluate the distribution and management options of Parthenium around Gondar town. The study was conducted in around Gondar town across the peripheral roads. The study consists of field survey and evaluation of management options and it was purposeful cross sectional experimental and with quantitative study. The whole plant bioassay, leaf detached bioassay and seed germination bio assay tested by species of fungi isolated from the leaves of Parthenium weed and chemicals (Glyphosate and 2, 4-D). The most effective fungus spp. was *Alternaria alternate* that induced 83% leaf damaged area on the 7<sup>th</sup> day in both leaf detached bioassay and whole plant bioassay. On the seed germination bioassay the most effective was *Aspergillus niger* (f1) that reduce the seed germination by 76.5% followed by *Alternaria alternate* (f4), *Fusarium solani* (f2), *Fusarium pallidoroseum* (f6), *Gliocladium virens* (f3), *Colleotrichum gloeosporioides* (f5), 2,4-D and Glyphosate that inhibit seed germination by 71.28%, 68.23%, 64%, 60.33%, 58.5%, 39% and 38.33% respectively. The importance of this study was to introduce the management options of Parthenium weed and to decrease the impact of Parthenium weeds. The study concluded that biological management options of Parthenium weed was more effective than chemical herbicides.

## KEY WORDS:

Biological control, Chemical control, Distribution of Parthenium weed and Mycoherbicide

## I. INTRODUCTION

Parthenium (*Parthenium hysterophorus* L.) weed is an annual herb of Asteraceae family, originating from tropical Americas and now a weed of global significance in many countries around the world. It is an exotic, pernicious weed is considered as one of the most troublesome weeds for agricultural sector by virtue of its high ecological amplitude and adaptability. It causes asthma, dermatitis, eczema and hay fever in humans and dermatitis in animals (Kaur *et al.*, 2016). Physical methods of control of this weed include manual de-weeding before flowering, as de-weeding after flowering leads to increased seed dispersal and germination (Author, 2011), harbors a diversity of pathogenic fungi, as shown recently by (Aggarwal *et al.*, 2016). It is an allelopathic weed and it inhibits the germination and growth of several crop plant and trees. The allelopathic potential of *Parthenium hysterophorus* weed result from the release of phytotoxic substances such as ferulic, caffeic, vanillic, chlorogenic, parthenin, ambrosin and coronopillin (Ramanujam *et al.*, 2015). According to El-darier (2015), Australian Parthenium weed seeds can germinate in a wide range of temperatures, including those as low as 10°C or as high as 36°C.

The allelopathic potential of Parthenium weed results from release of phytotoxic substances such as ferulic, I chlorogenic, p-coumaric and p-hydrobenzoic acids, parthenin, amhrasin and coronopillin, which inhibit the germination growth and of several crop plants and multipurpose trees (Nagaraja and Deshmukh, 2009). So far, most research on *P. hysterophorus* has focused on its chemical and biological control while knowledge about its regeneration biology is rather limited (Saxena and Kumar, 2007) and in some respects conflicting. For instance, studies conducted on the longevity of *P. hysterophorus* seeds have given inconsistent results. Nagaraja and Deshmukh (2009) reported germination decline from 66% after 1 wk of burial to 29% after burial for 2 yr. However, Navie *et al.* (1998) reported 74% seed viability after 2 yr's burial and the predicted half-life of the seeds to be about 6 yr (Tamado *et al.*, 2002).

With increasing societal concern regarding the harmful effects of chemical pesticides on humans as well as on environment, ecofriendly alternatives are being extensively sought. A variety of weed diseases are caused by fungi which have a potential to be used as bioherbicide or mycoherbicides (Saxena and Kumar, 2007). Fungi causing diseases in weeds are a diverse assemblage of species that markedly differ in their morphological and physiological characteristics. Further strains of a single species may vary in its pathogenicity (Saxena and Kumar, 2007). Today *Parthenium* has a position among the list of top ten worst weeds of the world (Holm *et al.*, 1977) and has been listed in the global invasive species database. Parthenium is not only a major threat to agricultural or horticultural system but is also a potential hazard to livestock and humans (Basak 1984; Valiappan and Towers 1989; Swaminathan *et al.*, 1990). There are different researches already done internationally and locally about *Parthenium* weeds and other invasive species in Ethiopia. Ayele *et al.* (2012) showed the distribution and the impact of *Parthenium* weed (*Parthenium hysterophorus*) in southern Ethiopia and a few survey was undertaken about the potential and actual invasive alien species and their biological threats to bio diversity in Awash National Park (Park, 2011). Another research to overcoming the challenge of *Parthenium hysterophorus* through composting and control (Araya *et al.*, 2015). Even though *Parthenium hystophorus* is among the top highly targeted weed in the weed management program of the research institute of Ethiopia, some areas are still left untreated and there is no specific studies on the newly expanding, diversity and controlling methods. So, the importance of this study is to explore the distribution, harmful and beneficial effect as well as the possible management practice against Parthenium weed and to enhance the quality of life for human being and livestock. The main objective of this study is to assess and evaluate the potential of fungal agents against Parthenium weed in related to chemical herbicide.

## II. MATERIALS AND METHODS

### Study area and study period

The study was conducted in around Gondar town, located in the north western of Ethiopia with latitude and longitude is 12.60 and 37.47 respectively (PCGN Romanization of Amharic, 2008). Its elevation 2072 and 2157 meters above sea level. The annual mean temperature is 19.1 degrees Celsius. Total annual Precipitation averages from 1115 to 1356 mm (Gondar climate- data organization, 2008). The average minimum and maximum temperature of the town is 9.8 C<sup>o</sup> to 29.7 C<sup>o</sup>. The studies was conducted from November, 2016 to May, 2017.

### Study design

The study consists of purposeful field survey and evaluation of management options and it was purposeful laboratory experimental and with quantitative study.

### Sampling Procedure of Survey

#### Sample sites and data collection

The sample sites were Azezo, Dupo and around Gondar University main campus. The field study was under taken between March, 2017 and April, 2017. Road transect survey was employed according to Wittenberg *et al.* (2004). Fifteen sample quadrates each with 2m x2m (4m<sup>2</sup>) were selected at every 20 m from each other in order to collect herbaceous data mainly Parthenium. A total of forty-five quadrates selected from road sides and farm lands. The infestation of Parthenium weed was estimated by counting the number of individuals per quadrates in relation to other herbaceous plants. The surveying study after collected the field data parameters like relative frequency and relative density were calculated by using the formula given below (Jai *et al.*, 2014).

Density = Number of individuals / area sampled

Relative Density = species density / total density for all species x 100

Frequency = Number of quadrates in which species occur / total # of quadrates sampled

Relative Frequency = species frequency / total of frequency values for all species x 100 (Jai *et al.*, 2014).

### Sampling procedure of experimental study

The sample sites were selected and then observed the leaves that had sign of diseases. The diseased leaves were brought to laboratory by sterile plastic bag. The leaves of Sixty Parthenium weed twenty from each sampling site were brought to laboratory and kept in appropriate place for the experiment. The sample was brought in to laboratory by plastic bag and kept in 4°C incubator for seven days for further experiment. The leaves were cut in pieces of 5 mm diameter and disinfected by 70% ethanol for three minutes and washed by distilled water and disinfected by mercury chloride for ten minutes and washed by distilled water three times. Pieces of leaves were placed on petriplate with fresh PDA. They were incubated it at 26± 2°C for a period of 9 days. Sub culturing was made in order get pure colony. The fungi was characterized and identified by spore morphology, microscopic examination and bio chemical test. Six different fungus species were isolated; *Aspergillus niger*(F2), *Fusarium solani*(F2), *Fusarium pallidoseum*(F3), *Alternaria alternate*(F4), *Gliocladium virens*(F5) and *Colleotrichum gloeosporioides*(F6) The conidia were harvested by flooding the Petri dishes with sterile distilled water (SDW). The conidial suspension was then filtered through muslin cloth to remove the mycelia bits. The leaf detached bio assay, the whole plant bioassay and seed germination bio assay were tested by different spore concentrations of fungi inoculums and different concentration of both Glyphosate and 2, 4-D. The experimental

study data was analyze by using one way ANNOVA by using SPSS ver.20 software to determine the means of the experiment and for any significant difference between the experimental sets.

Efficacy test =  $\frac{\text{seed germination in control} - \% \text{seed germination by treatment}}{100}$

Seed germination in control

Efficacy test =  $\frac{\% \text{ of health leaves in control} - \% \text{ health leaves in treatment}}{100}$

Percentage of control

$\% \text{ of health leaves} = 100\% - \% \text{damaged leaves}$

#### Leaf detached bioassay

The experiment was carried out as per method developed by chaing *et al.* (1989). Leaves of Parthenium weed were excise from shoot from the selected site (field). Three replicates comprising of four leaves each were tested and these replicates were sprayed evenly with five mile liter of different fungal inoculums using sprayer and also sprayed by ten mile liter of two different chemicals (glyphosate and 2, 4 -D) and was placed in moist chamber using sterile forceps. Three replicate comprising of four leaves served as control. Treatment was carried out within 20 min after detachment from the mother plant. All treatment were kept in growth chamber with control condition of  $26 \pm 2^\circ\text{C}$ ;  $75 \pm 15\%$  relative humidity and 15h illumination for a period of 7 days. Leaves were rate for diseases severity every 48 hour for seven days as per Chaing *et al.* (1989).

#### Whole plant bio assay

One hundred fifty seeds of Parthenium weed were collected from the field and disinfected with 70% alcohol for three minutes and washed with distilled water and then disinfected by mercury chloride for ten minutes and washed by distilled water three times (Tanvir, 2013). The seeds were sown in soil with 2.5 kg capacity pots. Five seeds were sown in each pot. The plant grown for seven weeks and they were sprayed with 100 ml fungal inoculums of different genus and species that prepared in the lab and 100 ml different chemicals (2,4-D and glyphosate) . i.e. the average number of damaged leaf were calculated and changed to percentage. The experimental set had three replicates each and the control set had three replicates. The control set was received only SDW. These were covered with plastic bags and kept in controlled conditions as described previously. After 24 h, the bags were removed and Plants were observed daily for the disease severity as per Chaing *et al.* (1989) until all plants died. The experiments were repeated three times.

#### Seed germination test

One hundred fifty seeds from the matured plant of the weed on the sampling site were brought in to the University Gondar Micro biology Laboratory. The plant seeds were surface sterilized by dipping in 0.05 %  $\text{HgCl}_2$  solution for 10 minute and then washed in tap water .Five seeds were placed on Petri dish on germination paper. Ten ml of different of fungal spore was add for biological agent and ten ml of chemicals (2, 4-D, glyphosate). The Petridis was incubated at  $22^\circ\text{C}$  with 70% humidity in dark for fourteen days .Distilled water serve as control .

#### Data to be collected

For both studies quantitative data were collected .in experimental study morphology, shape change of color, average number of leaf damaged area, average number of seed germination were collected. For the surveying study the altitude, latitude and slope of each sampling site were collected. The average cover abundance; relative frequency and relative density of Parthenium weed were also being collected.

#### Data analysis

The experimental study was analyzed by using SPSS version 20 software. The field study was analyzed by using descriptive statistics.

### III. RESULT AND DISCUSSION

#### Field study

In this study the different sites showed different level of infestation of *Parthenium hysterophorus*.). The data showed at site- II contained Parthenium plants 98 followed by site I and site- III with a number of 80 and 78 individuals respectively .The highest sociability of Parthenium weed was observed at site II with coverage and frequency of Parthenium weed of 89.9 %and 93.3% respectively followed by site I having a sociability of 52.5 and 85.1 respectively..

Table 1-The distribution of Parthenium weed at three sites of Gondar towns.

| Site | Name of plant                   | Total no. of individual spp. | Total no. of quadrat which spp. occur | Total no. of quadrat studied | Coverage of the spp. In % | Frequency in % |
|------|---------------------------------|------------------------------|---------------------------------------|------------------------------|---------------------------|----------------|
| I    | <i>Parthenium hysterophorus</i> | 80                           | 12                                    | 15                           | 85.1                      | 80             |
|      | Other plants                    | 14                           | 3                                     | 15                           | 14.9                      | 20             |
| II   | <i>Parthenium hysterophorus</i> | 98                           | 14                                    | 15                           | 89.9                      | 93.3           |
|      | Other plants                    | 11                           | 1                                     | 15                           | 10.1                      | 6.7            |
| III  | <i>Parthenium hysterophorus</i> | 78                           | 12                                    | 15                           | 96.2                      | 80             |
|      | Other plants                    | 3                            | 3                                     | 15                           | 3.8                       | 20             |

Where site I Dupo, site II around University of Gondar and site III Azezo

#### Detached Leaf Bioassays

Six species of Fungus was isolated from leaves of parthenium weed and identified using morphological structure, microscopic and biochemical test(fermentation of eight different carbohydrates. *Aspergillus niger*, *Fusarium solani*, *Fusarium pallidoseum*, *Alternaria alternata* , *Gliocladium virens* and *Colleotrichum gloeosporioides* were the species of fungi that isolated from leaves of Parthenium weed.

The initial symptoms of the disease started developing after 72 hours post treatment (hpt) in six test sets which received six different fungal isolates , f1, f2, f3, f4 , f5and f6 and two chemicals; glyphosate and 2,4-D (Table-2). The maximum average leaf area damaged was83% followed by 81%and 80% exhibited by f4,f5 and f2 respectively after seven day post treatment.

The best fungus species(F4) that were selected for further evaluations using whole plant bioassay was the one that inducing maximum average leaf area damaged of 83% on seventh day and 52% by third day. One way ANOVA indicates a significance difference the mean leaf area damaged at day five and seven with p-value  $p < 0.01$ .one way ANOVA indicates that f4 is more effective than others when comparing with control group.



**Table 2-diseases development of six different fungus and two different chemicals by leaf detached bio assay on different day after treatment**

| Fungal isolate(code) | Day three                | Day five                  | Day seven                  |
|----------------------|--------------------------|---------------------------|----------------------------|
| F1                   | 12.67%±1 <sup>d</sup>    | 42.67%±1 <sup>d</sup>     | 43.67%±1 <sup>d</sup>      |
| F2                   | 49.42%±5 <sup>b</sup>    | 79.42%±5 <sup>**b</sup>   | 80.42%±5 <sup>**b</sup>    |
| F3                   | 45.17%±17 <sup>b</sup>   | 75.17%±17 <sup>**b</sup>  | 76.17%±17 <sup>**b</sup>   |
| F4                   | 52.67%±8 <sup>a</sup>    | 82.67%±8 <sup>**a</sup>   | 83.67%±8 <sup>**a</sup>    |
| F5                   | 51.50%±14 <sup>a</sup>   | 80.17%±14 <sup>**a</sup>  | 81.0000%±14 <sup>**a</sup> |
| F6                   | 40.2500%±15 <sup>c</sup> | 68.7500%±15 <sup>*c</sup> | 69.7500%±15 <sup>**c</sup> |
| 2,4-D                | 38.0000%±18 <sup>c</sup> | 70.0000%±15 <sup>*c</sup> | 71.17%±14 <sup>**c</sup>   |
| GLYPHOSATE           | 43.67%±8 <sup>b</sup>    | 72.17%±8 <sup>*b</sup>    | 73.17%±8 <sup>**b</sup>    |
| CONTROL              | —                        | —                         | —                          |
| LSD                  | 0.117                    | 0.45                      | 0.445                      |

Where, \*\* indicates significant in both  $p \leq 0.01$  and  $p \leq 0.05$  and different letters indicate LSD values at  $p < 0.05$ .

#### Whole plant bioassay

During the *whole plant* bioassay on whole plants it exhibited onset of disease after three days post treatment and 43.67%- 83.67% death by seven days post treatment. Fifty per cent damage of the weed occurred after three day (Table 4). A similar trend was observed in the Detached Leaf Bioassay where

42.67%- 82.67% Mortality occurred after five day and chemicals exhibit mortality on seventh day 71.17%-73.17%. There was no significant change in the means of the replicates of the experiment on third day as indicated by the P value of one way ANOVA.

**Table 3-Average number of damaged leaf in percentage in the whole plant bioassay**

| Treatment  | Day three                | Day five                 | Day seven                  |
|------------|--------------------------|--------------------------|----------------------------|
| F1         | 2.67%±1 <sup>d</sup>     | 42.67%±1 <sup>d</sup>    | 43.67%±1 <sup>d</sup>      |
| F2         | 39.42%±5 <sup>b</sup>    | 79.42%±5 <sup>**b</sup>  | 80.42%±5 <sup>**b</sup>    |
| F3         | 35.17%±17 <sup>b</sup>   | 75.17%±17 <sup>**b</sup> | 76.17%±17 <sup>**b</sup>   |
| F4         | 42.67%±8 <sup>a</sup>    | 82.67%±8 <sup>**a</sup>  | 83.67%±8 <sup>**a</sup>    |
| F5         | 41.5000%±14 <sup>a</sup> | 80.17%±14 <sup>**a</sup> | 81.0000%±14 <sup>**a</sup> |
| F6         | 30.2500%±15 <sup>c</sup> | 68.75%±15 <sup>*c</sup>  | 69.7500%±15 <sup>**c</sup> |
| 2,4-D      | 31.33%±15 <sup>c</sup>   | 70.00%±15 <sup>*c</sup>  | 71.17%±14 <sup>*c</sup>    |
| Glyphosate | 33.67%±8 <sup>b</sup>    | 72.17%±8 <sup>*b</sup>   | 73.17%±8 <sup>*b</sup>     |
| Control    | —                        | —                        | —                          |
| LSD        | 0.501                    | 0.45                     | 0.448                      |

Where, \*\* indicates significant in both  $p \leq 0.01$  and  $p \leq 0.05$  and different letters indicate LSD values.

According the LSD values(F4 and F5 were the highest and F1 and 2, 4-D were the lowest. But the chemical herbicides 2, 4-D and glyphosate were compared with the six different fungus species those two different chemicals are less than fungi species except F1.

#### Seed germination bioassay

The effects of six different fungal isolates and two different chemicals on germination percentage (GP) were represented in Table 4. The data demonstrated that the GP was significantly affected by applying the different fungus species and chemicals. More obvious reduction (76.5%) in GP was seen when treated by f1. The reduction percentage at glyphosate and 2, 4-D was 38.33%.

**Table 4-The effect of six different fungus and two different chemicals on the percentage of seed germination.**

| Treatment  | Day seven                 | Day fourteen               |
|------------|---------------------------|----------------------------|
| F1         | 4.0%±2 <sup>**a</sup>     | 9.00%±2 <sup>**a</sup>     |
| F2         | 12.27%±4 <sup>**a</sup>   | 17.27%±4 <sup>**b</sup>    |
| F3         | 20.17%±10 <sup>**b</sup>  | 25.0000%±10 <sup>**c</sup> |
| F4         | 9.22%±4 <sup>**a</sup>    | 14.22%±4 <sup>**a</sup>    |
| F5         | 22.0000%±9 <sup>**b</sup> | 27.0000%±9 <sup>**b</sup>  |
| F6         | 16.5%±11 <sup>**a</sup>   | 21.50%±11 <sup>**a</sup>   |
|            | 41.5000%±20 <sup>c</sup>  | 46.67%±20 <sup>c</sup>     |
| Glyphosate | 42.17%±4 <sup>c</sup>     | 47.17%±4 <sup>c</sup>      |
| Control    | 80.5000%±2                | 85.5000%±2                 |
| LSD        | 1.00                      | 1.00                       |

Where, \*\* indicates significant in both  $p \leq 0.01$  and  $p \leq 0.05$  and different letters indicate LSD values.

According the results in seed germination bioassay F1 followed by F4 were the highest and glyphosate and 2, 4-D were the lowest. All fungus species treatments showed higher than both chemical herbicides.

**Table 5-Effectiveness of six different fungal isolates and two different chemicals on seed germination bio assay.**

| Treatment  | Day seven | Day fourteen |
|------------|-----------|--------------|
| F1         | 95%       | 89.4%        |
| F2         | 84.75%    | 79.8%        |
| F3         | 74.9%     | 70.7%        |
| F4         | 88.5%     | 83.3%        |
| F5         | 72.6%     | 68.4%        |
| F6)        | 79.5%     | 74.8%        |
| 2,4-D      | 48.4%     | 45.4%        |
| Glyphosate | 47.6%     | 44.8%        |
| Control    | -         | -            |

In both whole plant bioassay and detached leaf bioassay *Alternaria alternata* was shown Effectiveness in killing the weed shoot sytem. According to the effectiveness of six of six different fungal isolates and two different chemicals on seed germination Bioassay F1 followed by F4 were the highest and glyphosate and 2,4-D were the lowest. Almost all fungus species shown higher efficacy results than both chemical herbicides.

The present study shows that Parthenium weed is very dominant in road sides. This result was in agreement with studies by Length (2013), show heavy and widespread infestation mostly on roadsides. The study shows *Parthenium hysterophorus* showed a high degree of socialty. This result is in agreement with Knox *et al.* (2011) conducted a phytosociological survey in the wastelands of Raipur district during the rainy season. He recorded about 27 weed species associated with *P. hysterophorus*. Among all weeds, *P. hysterophorus* L. showed a high degree of sociability and formed into large colonies under arable soil habitats. It indicates the action of six different fungus species and two different chemical herbicides on the management of Parthenium weed agreed with Lahore (2004) indicated that biological management option of Parthenium weed have played a significant role in the inhibition of the groth of and seed germination. The overall effect of six different fungus and two different chemicals on the germination and growth of of Parthenium weed agreed with Shafique *et al.* (2011) described that the strong relationship between different concentration of fungus species and toxicity to Parthenium weed. The fungal pathogens of Parthenium weed were screened qualitatively and quantitatively for the managing of Parthenium weed agreed with Tanvir *et al.* (2013) suggested that

the fungal flora isolated from leaves of *P.hysterophorus* from different locations consisted mostly saprophytic fungi. The present study suggested that most of the isolated showed pathogenic effects on Parthenium weed leaves and seeds. The study indicated that *Alternaria alternata*(F4) is the most effective according the means of leaf damaged area percent both leaf detached bio assay and whole plant bioassay agreed with Saxena et al. (2007) suggested *A. alternata* an indigenous isolate of *Alternaria* at spores concentration induced nearly 90% leaf damage. One way ANOVAs indicates a significance difference between the mean leaf damaged area by different fungus species with a P value of  $p < 0.05$  and  $p < 0.01$ . The present study indicated *Alternaria alternata* seem to offer great potential for development effective diseases on the leaf of Parthenium weed agreed with Evans (1997) reported that leaf spot diseases caused by *Alternaria alternata* was associate with Parthenium weed damage. The study investigated the action of biological herbicides on some germination considerations and seedling growth of *P.hysterophorus* under laboratory conditions agreed with El-darier (2015) indicated that mycoherbicides inhibitory effects on seed germination, leaf detached bio assay and whole plant bio assay. To go through with this, leaf area index were inhibited in all treatment when compared with the control. Bioherbicides can play significant role in the Management of Parthenium weed in agreement with Evans (1997). Fungal biocontrol agents should have a rapid life cycles and cause damage to the host plants.

### III. CONCLUSION

The study showed that Parthenium weed around Gondar town was very dominant. The six fungus species and two chemicals had a significant on seed germination ,whole plant bioassay and detached leaf bioassay .but the degree susceptible between fungus species and chemicals was different .i.e. fungus species were most effective. The present study showed that *Alternaria alternata* has highest significant activity against Parthenium weed. The present study showed that biocontrol fungal species have significant potential herbicidal effect on monitoring the germination and growth of Parthenium weed. The study recommended that Management of Parthenium weed biological management option is a good ecofriendly herbicide application. Care must be done to control the current expanding Parthenium weed around Gondar and the surroundings. Molecular characterization of the biocontrol should be the future work. Formulation and mass cultivation is also another future activity for commercialization.

### IV. ACKNOWLEDGMENT

We would like to thank University of Gondar Research and publication office financial grant, for Mega project from “Sustainable production of healthy seedling systems for rice, pepper and tomato for small scale farmers in northern west Ethiopia project” to complete the study.

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#### Corresponding author :

**Samuel Sahile \***

College of Natural and Computational Science, University of Gondar, P.O.Box 309, Gondar, Ethiopia.  
Email : hanasahile[at]yahoo.com

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