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Inhibitory Activities of Sole Cow Urine and Combined Cow Dung/Cow Urine against the Blight Disease of Ribwort (*Plantago lanceolata*) at the Cistercian Monastery in Mbengwi, Cameroon

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Abstract

Ribwort (*Plantago lanceolata*) is a small glabrous to pubescent perennial plant that is native to Europe, America, North Africa and Asia. Nowadays, it is cultivated in many countries across the globe, including Cameroon due to its extensive use in livestock and medicine. Unfortunately in Cameroon, however, the plant has been highly infested by blight, reducing its yield and medicinal value. To reduce blight infestation of ribwort and improve plant yields, we aimed to compare the efficacy of sole cow urine and combine cow dung/cow urine to inhibit blight disease caused by *Phyllosticta ophiopogonis* on ribwort. At the Cistercian Monastery in Mbengwi, Momo Division, Cameroon, we used a Randomized Complete Block Design (RCBD) with 3 Blocks consisting of two treatments (*cow dung mixed with cow urine* (*combine cow dung/urine*) *and sole cow urine*) and *one control*. After spraying the different blocks of ribworts plants with combined cow dung/urine and sole cow urine at a dosage of 3% concentration, we found an incidence of blight disease of 32.8% and 35.0% on ribworts sprayed with combined cow dung/urine and sole cow urine, respectively, compared to 67.8% in the control. This implies that a mixture of cow dung/cow urine reduces the incidence of blight disease significantly. Furthermore, our pathogenicity test showed that *Phyllosticta ophiopogonis* (*fungus*) was responsible for the blight disease. Therefore, to increase ribwort growth, improve adaption and reduce *Phyllosticta ophiopogonis* fungal infestation in Cameroon, we recommend that the plant should be sprayed with a mixture of cow dung and cow urine at 3% concentration.

Keywords

Cow Dung and Cow Urine, Inhibitive Activity, Ribwort, *Phyllosticta ophiopogonis*

1. Introduction

Ribwort is a plant that has existed for nearly 4000 years in Europe, America, North Africa, and Asia [1] [2]. However, the plant is now cultivated in many countries across the globe, including the continental states in the United States of America (USA), Hawaii, Australia, New Zealand, Japan, and Africa, especially in areas with high altitude [3]. Ribwort (*Plantago lanceolata*) (**Figure 1(a)**) is a small, glabrous to pubescent perennial plant with one to several rosettes. The plant has linear to narrow ovate-elliptic leaves, measuring about (2 - 30) × (0.5 - 3.5) cm. It also has a slender petiole that is separated by midribs and bracts measuring 2.5 - 3.5 mm. The inflorescence is a spike measuring up to 4 cm long. Ribwort has bisexual flowers that are inconspicuous with 4-lobed corolla which are tubular, and almost as long as the surrounding calyx. The plant also has 4 conspicuous stamens of about 3 - 5 mm long, including yellowish anthers. The fruits are 3 - 4 mm long, and the capsule opens with an operculum that contains 1 - 2 smooth, boat-shaped mucilage [4] [5].

Ribwort is used extensively in agriculture and to manage a wide range of diseases. Firstly, ribwort is used as pastures for animals and for sward improvement [6] [7]. Secondly, the commercial cultivars, grasslands Lancelot, and ceres tonic have been developed in New Zealand for forage yield and livestock grazing [8] [9]. Thirdly, the plant is used to treat respiratory, urinary and gastrointestinal diseases and to manage stings, boils, tumors, inflammations, eczema, thrush, poisoning,

(a) (b)

Figure 1. (a) (Health Ribwort) [20]; (b) (Ribwort infested by blight).

ulcers, bleeding wounds, diarrhea, piles, scratches, burns, bed-wetting, syphilis, toothache, worms, running sores, and ringworm [10]. Additionally, the roots have been used in combination with horehound (*Marrubium vulgare*) to manage rattlesnake bites while the seeds have been used to treat parasitic worms [11].

Due to its medicinal value, ribwort was introduced in Cameroon a few years ago at the Cistercian Monastery in Mbengwi. Unfortunately, the plant has been facing numerous adaptability problems, including blight diseases that have continued to reduce its yield and medicinal value. Pathogens, including fungi, attack the tissues, harm the secretory cells, and lead to the modification of the biologically active substances within the plant [12]. In Poland, for instance, studies conducted on the different types of fungi that colonize and damage selected plant parts of ribwort found that *Phyllosticta plantaginis* affects the leaves, causing small, regular, necrotic spot [13].

The use of bio-enhancers in agriculture such as cow dung and cow urine is an age long practice that has been used to enrich soils, control pests and induce better plant vigour. These organic waste products from cows are rich sources of microbial consortia, micronutrients, plant growth promoting substances and immunity enhancers [14]. Studies conducted in Indian subcontinent where cow by-products are held in high esteem, have shown that cow by-products have bioactive properties that make them potent antibacterial, antioxidant and antifungal [15]. The antifungal activity of cow urine has been confirmed against Aspergillus spp, and Candida spp [16]. When used in combination with some botanical extracts, waste products from cows such as cow urine effectively inhibited the growth of Rhizoctonia spp., Fusarium spp., Sclerotinia spp., Phytophthora spp and Colletotrichum spp [17].

At the time of conducting this research, no similar study has been done on ribwort in Cameroon, mindful of the exotic nature of the plant. Therefore, we aim to study the efficacy of sole cow urine and a mixture of cow dung/cow urine against blight disease of ribwort to improve the production and to maintain the medicinal value of the plant in Cameroon.

2. Materials and Methods

2.1. The Study Site

The research was conducted at the Cistercian Monastery in Mbengwi, Momo Division, an area that falls within the Agro-ecologic zone III of Western Highlands, in the North West Region of Cameroon. The area has an altitude of 900 m to 2000 m above sea level. Mbengwi is located at longitude 100˚E to 100.02˚E and latitude 6.05˚N of the equator. It also has an annual rainfall of 2189 mm and yearly temperature of 21.4˚C.

2.2. Experimental Study Design

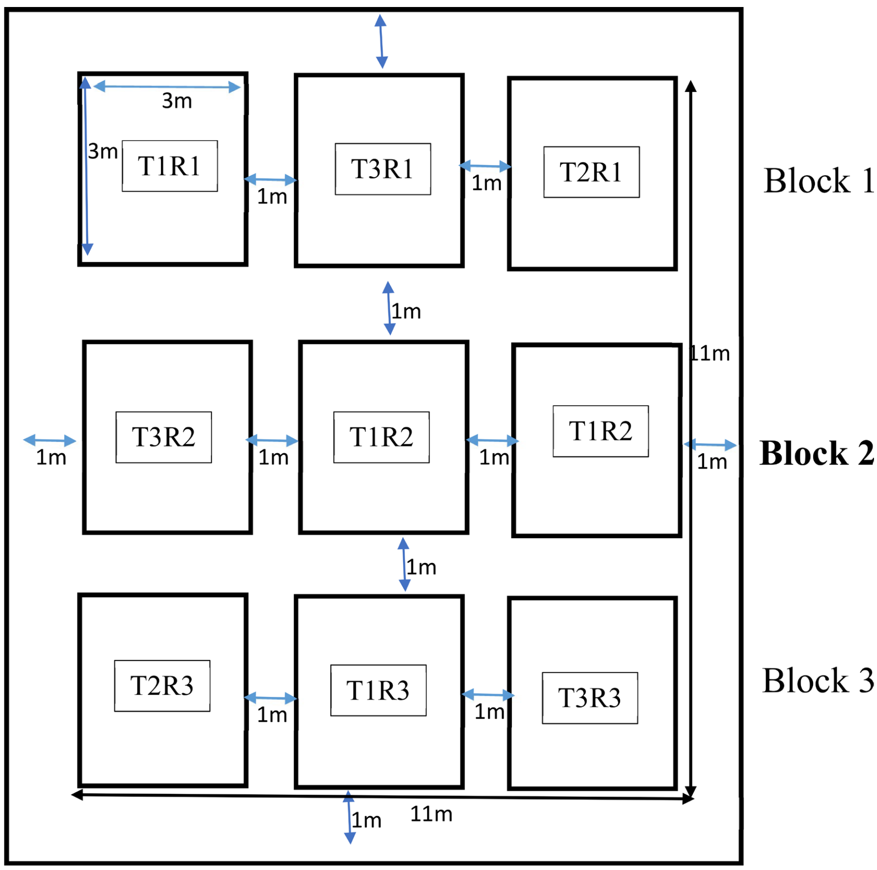
We used a Randomized Complete Block Design (RCBD) with 3 blocks and 3 treatments, making a total of 9 treatments. The experimental farm surface area was 121 m^2, with a distance of 1 m between blocks and treatments. 100 g of cow dung manure was applied per stand. The layout and design of the pecking and forming of ridges are as shown in Figure 2.

Experimental Unit

The experimental unit of the plot measured 4 m × 3 m (12 m^2) and consisted of 5 ridges with 6 plants per ridge, giving a total of 30 plants per unit. The intra-row distance of the plants was 50 cm, and the inter-row distance of the plant was 60 cm. However, the response unit (*i.e.*, the shaded area) was demarcated to avoid border effects (Figure 3).

2.3. Planting and Cultural Practices

We transplanted the uninfected ribwort suckers from the herbal garden at the Cistercian Monastery in Mbengwi. They were planted at a depth of 5 cm and at distances of 60 cm inter-plant and 50 cm intra-plant spacing. Weeding and mulching were carried out at 4 weeks after planting (WAP), and the second weeding was done at 8 WAP.



Key words: T1 = Cow dung/cow urine; T2 = Cow urine; T3 = Control.

Figure 2. A schematic diagram showing the different treatments in the experimental plot.

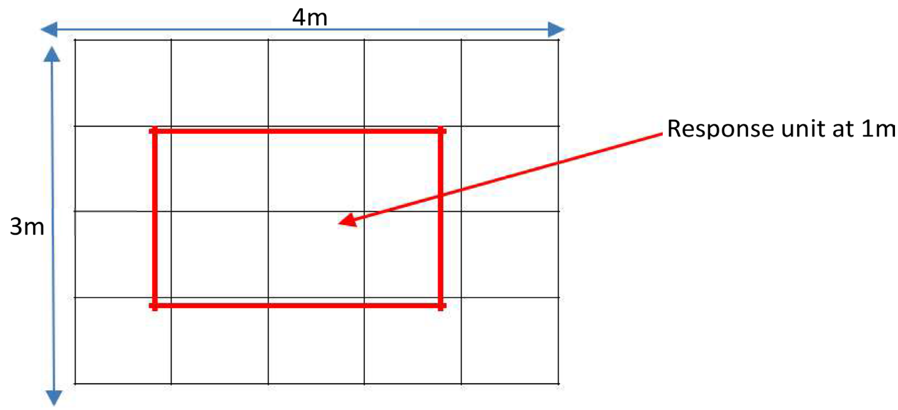


Figure 3. A schematic diagram showing the response unit.

2.4. Preparation of Extracts of Cow Urine and the Mixture of Cow Dung/Cow Urine

2.4.1. Cow Urine

Cow urine was prepared as follows: 10 liters of cow urine was collected in the morning from different cows (dairy cows) at the cow shade of Cistercian Monastery in Mbengwi and put in the same container and swirl. The cow urine was kept in an airtight container to ferment for 2 weeks. One hundred grams (100 g) of powder soap (omo) was added as a surfactant, and the entire mixture was placed in an airtight container [18].

2.4.2. Cow Dung/Cow Urine

The cow dung and cow urine extract were prepared as follows: 5 kg of fresh cow dung was collected from the cow shade at Cistercian Monastery in Mbengwi. The cow dung was mixed with 5 liters of waters in a ratio of 1:1. The mixture was put in an airtight container and allowed to ferment for 15 days. This was then strained after 15 days with a cheesecloth, during which 5 liters of it was mixed with 5 liters of fermented cow urine. One hundred grams (100 g) of powder soap (omo) was added as a surfactant, and the mixture was put in an airtight container [18].

2.4.3. Dosage and Control Measures

The prepared extracts were applied at 3% concentration on the crops as previously described by Natarajan [19], that is, 30 ml of extract diluted into a liter of water = 3% concentration. The preparation was used to spray the plants against blight at 3 WAP, following the appearance of symptoms of blight on the plant. The spraying was done twice a week at an interval of 3 and 4 days. This interval was adopted because the experiment was laid out during the rainy season. If the experiment was carried out in the dry season, then spraying would have been done once a week.

2.5. Source of Inoculum

Inoculation was used to introduce the disease in the field. Inoculation was done in the evening to ensure proper establishment between the plant and the pathogen enhanced by the nocturnal dew. The process was as follows: The diseased plants were harvested from the experiment site, mixed with water and macerated. The extract was sprayed on the surface of the plant across different blocks except for the control.

2.6. Data Collection for Disease Incidence

Percentage disease incidence was monitored on the reposed area of each plot (each plot contains 12 plants). The diseased plants were assessed weekly on all the treatments and recorded. The percentage of disease incidence was calculated using the formula below.

$$\text{\% of Disease Incidence} = \frac{\text{number of plants infected/plot}}{\text{Total number of plants sampled/plot}} \times 100$$

2.7. Pathogenic Testing of *P. ophiopogonis*

2.7.1. Preparation of V6 Juice Agar

A V6 juice agar was prepared as follows: 6 gms of green beans, 40 gms of garden pea, 10 gms of flageolets, 30 gms of carrot and 4 gms of taro leaves were measured using an electric balance. All were washed, boiled, and blended together with 500 ml of distilled water. The resulting solution was filtered twice with a muslin cloth, and 150 ml of marcedoine juice was then added. The solution was put in a conical flask, sterilized, and distilled water was added to make a solution of 1000 ml. 20 g of agar, 3 g of calcium carbonate, and 1 ml of vegetable oil were added to the mixture. The mixture was homogenized by swirling. The mouth of the flask was covered with non-absorbent cotton wool and wrapped with aluminum foil. The flask was put in an autoclave and sterilized at a temperature of 121˚C and a pressure of 1 atm, for 20 minutes. Ampicillin (250 mg/l), penicillin (250 mg/l) and nystatin (20 mg/l) were added to the culture media after sterilization. When the temperature of the media was cooled to about 40˚C, everything was well mixed. 20 ml of the media was poured into 10 sterilized Petri dishes as described by Manju and colleagues [21]. Growth was observed in the cultures within a period of 2 - 3 days, and the emerging fruiting bodies were described.

2.7.2. Collection, Isolation, and Identification of Fungi from Infected Plant Materials

The infected ribwort leaves with young lesions of the blight disease were collected from the farm at the Cistercian monastery in Mbengwi, placed in plastic bags and transported to the crop protection laboratory of the Institute of Agricultural Research for Development (IRAD) Bambui. At the advanced stage of the disease on the plants, the leaves were cut with a razor blade into small fragments of 2 mm and sterilized in 5% diluted solution of sodium hypochlorite for 30 seconds and rinsed in 3 successive changes of distilled water for 3 minutes. Fragments of the leaves were dried using a sterile filter paper and three pieces placed on solidified cold V6 juice culture agar medium in each Petri dish. The dishes were labeled (L1) with the date written on it and put in an incubator at a room temperature of 25˚C [21]. After 5 days, an extensive mycelial growth was formed around the fragments of the leave. Under aseptic conditions, the mycelial were collected using a cork borer and a wire loop and put in Petri dishes containing freshly prepared V6 juice agar and antibiotics that inhibit bacteria growth. It was subcultured, and after 5 days, they were extensive mycelia growth. The sub-culturing of blight disease was done twice to obtain an axenic culture or pure culture.

2.8. Statistical Analysis

The data collected from the ribwort infections were subjected to Analysis of Variance (ANOVA) [22], using statistical software STATGRAPHICS Plus 5.0. The mean values of the treatment were separated using the Least Significant Difference (LSD) for statistical significance at a 95% confidence interval ($P \leq 0.05$). Disease incidences over time were plotted for each treatment to monitor the progress of the disease. A comparative analysis was carried out on the treatments to determine the best inhibitive effects of the various treatments used to manage blight disease of ribworts.

3. Results

3.1. The Effect of Cow Urine against Blight Disease of Ribwort

The effect of cow urine against blight disease of ribwort showed some variations in the mean disease incidence in weeks after inoculation. A significant ($P \leq 0.05$) difference was observed in week 1 and week 2 compared to weeks 3, 4, 5, 6, 7, 8, 9 and 10, respectively. There was no significant difference observed in the mean disease incidence for the same treatment with weeks 3, 4, 5, 6, 7, 8, 9, and 10. In order of performance of cow urine in reducing blight disease of ribwort, there was a general decrease in the mean disease incidence with week 1 having the highest mean disease incidence, followed by week 2 and then week 3, and the rest of the weeks showing a lower mean disease incidence. Weeks 4, 5, 9, and 10 showed no significant difference in mean disease incidence, and this was the same for weeks 6, 7, and 8.

3.2. The Effect of Cow Dung/Cow Urine against Blight Disease of Ribwort

The effect of combined cow dung/cow urine mixture in the control of blight disease of ribwort differed significantly in mean disease incidence (**Figure 4**). A significant ($P \leq 0.05$) difference was observed with the effect of cow dung/cow urine in controlling blight disease of ribwort with the first week having the highest mean disease incidence after inoculation compared to weeks 2, 3, 4, 5, 6, 7, 8, 9 and 10 respectively. No significant difference was observed with cow

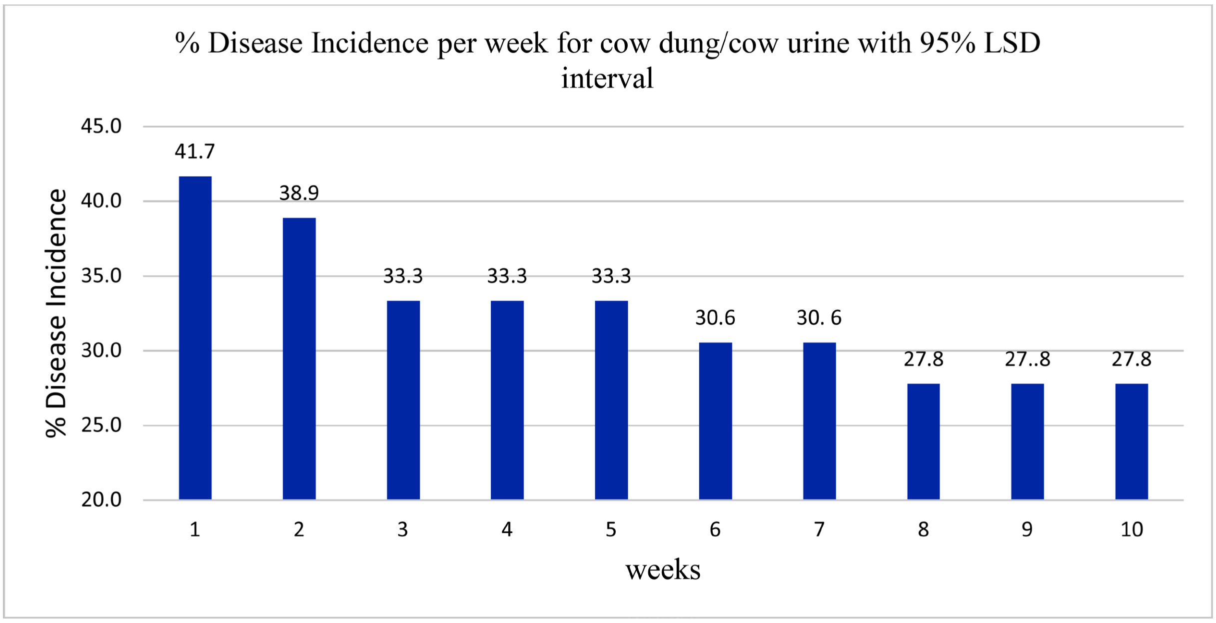


Figure 4. Effect of cow urine in the control of blight disease of ribwort at different weeks of observation.

dung/cow urine in the control of blight disease of ribwort at weeks 2, 3, 4, 5, 6, 7, 9 and 10 respectively; however, week 2 showed some variation in the mean disease incidence with a higher mean value. Also, no significant difference was observed between weeks 3, 4 and 5, and between weeks 6 and 7. This was the same with weeks 8, 9 and 10. When considering the performance of cow dung/cow urine in the control of blight disease of ribwort, it was observed that weeks 8, 9 and 10 showed the least mean disease incidence compared to the other weeks (Figure 4).

3.3. The Effect of Blight Disease of Ribwort on the Control Experiment at Different Weeks of Observation

The effect of blight disease of ribwort on the control experiment showed that there was an increase in mean disease incidence in all the weeks after inoculation (Table 1) in increasing order of weeks, with weeks 7, 8, 9 and 10 having the highest infection. Week 1 and week 2 showed no significant difference in mean disease incidence even though the incidence was higher in week 2. Also, no significant difference in mean disease incidence was observed with weeks 3, 4, 5, and 6, but weeks 5 and 6 had a higher mean disease incidence than weeks 3 and 4. Generally, the mean disease incidence on ribwort was higher in the control experiment when compared with sole cow urine and combined cow dung/cow urine.

3.4. Comparison of the Effectiveness of Cow Urine and the Mixture of Cow Dung/Cow Urine versus the Control Experiment against Blight Disease of Ribwort

In comparing the effectiveness of the two different treatments used against

Table 1. Effect of blight disease of ribwort on the control experiment at different weeks of observation.

Weeks	Blocks	Mean Disease Incidence
1	3	44.4c
2	3	52.8bc
3	3	63.9ab
4	3	63.9ab
5	3	72.2ab
6	3	72.2ab
7	3	77.8a
8	3	75.0a
9	3	77.8a
10	3	77.8a

Means in a column followed by the same letter are not significantly different at $P \leq 0.05$ (Using LSD).

blight disease of ribwort, it was observed that a combination of cow dung/cow urine reduces blight disease of ribwort, followed by sole cow urine, with the control experiment having the highest mean disease incidence compared to other treatments (**Figure 5**).

Sole cow urine and combined cow dung/cow urine treatments significantly reduced the incidence of blight disease of ribwort compared to the control. Even though the mixture of cow dung/cow urine reduced the mean disease incidence better than sole cow urine, the difference was not statistically significant.

3.5. Identification and Confirmation of the Blight Pathogen of Ribwort

From the pathogenicity test, the isolates of the fungi in an artificial culture media were consistent with that of the diseased leaves in pure culture, and it produced symptoms similar to that of the fungus group. The description of the spores, as observed under the microscope, showed that many were small and having black-colored pycnidia formed on the lesions. The conidia were unicellular, colorless, ovoid to oval, and surrounded with a thick mucilaginous sheath (**Figure 6(a)**). On the V6 juice agar, the colonies were utterly white with abundant mycelia, which were gradually becoming greenish to dark green after about 3 - 4 days with white hyphae on the undulate margin that later turn dark green to black. The colonies grew up to about 40mm in diameter on the V6 juice agar after 14 days (**Figure 6(b)**). The pycnidia appear on the colonies after 7 days of cultured. From the description of the growth of the fruiting body of the blight disease after the pathogenicity testing, we confirmed that the pathogen responsible for the blight disease of ribwort was *Phyllosticta ophiopogonis*.

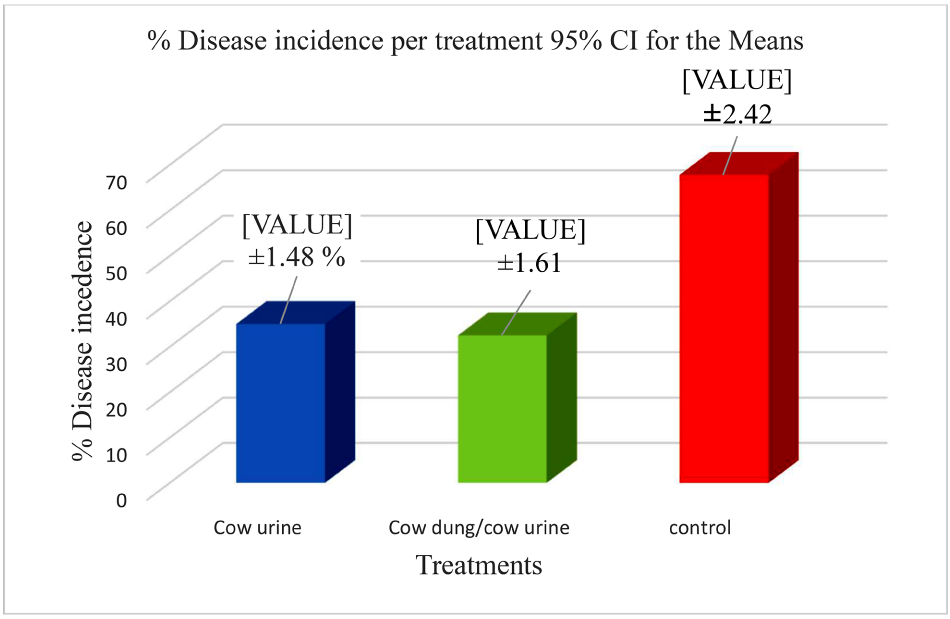


Figure 5. Comparison of the effectiveness of cow urine, cow dung/cow urine versus the control experiment against blight disease of ribwort.

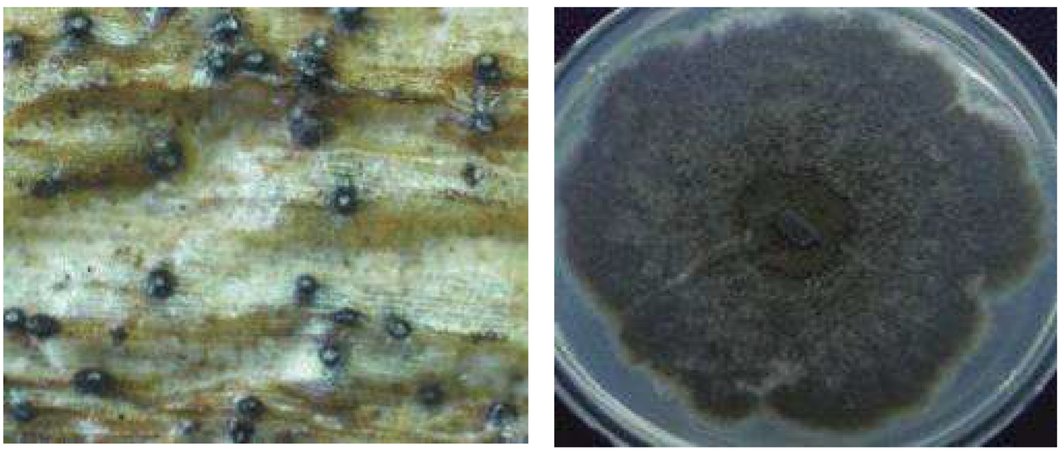


Figure 6. Morphological characteristics of *Phyllosticta ophiopogonis* (a) -pycnidia in infected leaf with conidia; (b) colony on V6 agar after 14 days of incubation (400×).

4. Discussion

Cow urine and cow dung have been used extensively in the control of plant diseases due to their antifungal and antiviral properties [23] [24]. These organic extracts have beneficial effects in the control of diseases and pests that affect plants and their seeds. Cow urine possesses antimicrobial activity against microorganisms that affect plants, including inhibitory activity against pathogens like *Sclerotinia sclerotiorum*, *Fusarium solani f. sp. Cucurbitae*, *Bipola rissorokiniana*, *Xanthomonas oryzae pv.* oryzae, *F. oxysporum f. sp. Zingiberi*, and *Colletotrichum capsici* [24] [25] [26] [27] [28]. Also, Sible and colleagues [29] reported a 50% reduction in bacterial leaf spot disease on rice leaves after 45 days of spraying with cow dung extract. However, in this study, we found that cow urine extract can reduce the incidence of blight disease on ribwort to 35.0% ± 1.61%, and a combination of cow dung/cow urine reduced the disease incidence rate further to 32.50% ± 1.48%. This shows that a mixture of cow dung and cow urine controls the infestation of ribwort by *Phyllosticta ophiopogonis* much better.

Cow dung has been used commonly in organic farming. In 2005, Charest and colleagues [30] showed that two bacteria (*Pseudomonas aeruginosa* and *Rhizobium radiobacter*) were present in cow dung manure, and they could act as inhibitors against blight disease on paddy rice. In 2011, Tiwari and Das conducted a research [31] and reported that foliar spray of cow dung on paddy rice could significantly reduce the incidence of blight disease by 18.5%. Likewise, sole cow urine in this research was effective in lowering blight disease of ribwort, and this is in agreement with previous studies by Hurali and Patil [32] who found that cow urine reduced the disease incidence of soybean rust by 36.0%. The growth performance of ribwort showed that despite the fungicidal properties of cow dung and cow urine extracts in controlling blight diseases, the extract could also serve as manure that helps to facilitate the growth of ribwort. The growth rate was higher in ribwort treated with cow dung/cow urine compared to those treated with sole cow urine. This implies that a combined cow dung/cow urine extract has more plant growth nutrients compared to sole cow urine. This suggests that cow dung is high in organic materials and rich in nutrients because it contains about 3 percent nitrogen, 2 percent phosphorus, and 1 percent potassium (3-2-1 NPK). Cow urine contains 95% water, 2.5% urea, and the remaining 2.5% is a mixture of minerals, salts, hormones, and enzymes [33]. In India, cow dung has been used tremendously in agriculture as manure, bio-fertiliser, biopesticides, pestrepellent, and as a source of energy [34].

5. Conclusion

Since the introduction of ribwort as an exotic agricultural and medicinal plant in Cameroon by the Monks of the Cistercian Monastery in Mbengwi, Cameroon, the plant has been facing numerous adaptability problems, including infestation by blight diseases leading to a reduction in its yields and therapeutic values. However, we found that cow dung and cow urine extracts significantly reduce the incidence of blight disease of ribwort. The decrease in the incidence rate of blight disease of ribwort was higher in plants treated with the mixture of cow dung and cow urine compared to sole cow urine. Our pathogenicity test confirmed *Phyllosticta ophiopogonis* as the pathogen responsible for blight disease of ribwort in Mbengwi, Cameroon. We also found that cow dung and cow urine are bio-enhancer that increases the nitrogen content of the soil and enhances plant growth. Therefore, to boost the growth of ribwort, improve its adaption and to reduce *Phyllosticta ophiopogonis* fungal infestation, we recommend that the plant should be sprayed with extracts from a mixture of cow dung and cow urine at a concentration of 3%.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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