Efficacy of *Ageratina adenophora* against White rust (*Albugo candida*): A laboratory and field study

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ABSTRACT

White Rust of Crucifers is caused by Albugo candida, which causes damage to quality and quantity of the produce. Conventionally, fungicides are applied to reduce disease incidences; however, improper and excessive use of chemical fungicides can be detrimental to the environmental health. In this study, different concentrations (2.5%, 5%, 7.5% and 10%) of freshly prepared aqueous extract of Ageratina adenophora were tested for antifungal activity in vitro and in vivo against A. candida using poison-food technique and Colony Forming Unit (CFU/ml). In vitro results revealed that 10% aqueous extract of A. adenophora was most effective against mycelial growth and biomass formation of A. candida.10% aqueous extract inhibited 92.7% of A. candida mycelial and total biomass formation followed by 78% at 7.5% concentration. The lowest inhibition was observed in 2.5% concentration with 2.4%. The in vivo antifungal activity of aqueous extract of A. adenophora was tested in the potted plants under normal conditions. The result revealed that the preventive control is most effective than the curative control. Preventive control of disease incidence using 10% aqueous extract led to 54.1% reduction, whereas the curative control reduced by mere 3.6%. Thus, 10% aqueous extract of A. adenophora demonstrates the potential for the control and management of white rust of crucifers.

Keywords: *Albugo candida, Ageratina adenophora, Aqueous extract, Antifungal activity, Curative control, Preventive control*

1. Introduction

Agriculture is the key source of sustaining livelihood and enhancing human life (Pino, Sánchez& Rojas, 2013). It supplies food and other essential commodities for human consumption. Apart from fulfilling food requirement of a growing population, agriculture plays pivotal role in improving the economy of a nation (Dutta, 2015). However, the damage and destruction inflicted on the crops by various pest and pathogens such as insects, microbes (bacteria, fungi, viruses and mycoplasmas), nematodes, weeds, animals and birds have posed serious challenges to farmers in terms of sustaining food productivity (Yadav, Kewal & Choudhary, 2015; Koul, 2011).

White Rusts (also called as white blister) are caused by several species of *Albugo*, belonging to the class Oomycetes. The disease attacks aerial parts of cruciferous plant including flower,

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leaves and stem. It does not attack the root of a large number of cultivated and wild crucifer plants (Sharma 2017). Cultivated plants which are susceptible to infection by white rust include cauliflower, cabbage, radish turnip, broccoli, and mustard. White rust caused by *Albugo candida* (Pers. ex. Lev.) Kunze (Saharan, Verma, Borhan & Singh, 2014) is an important and widespread disease in the world. According to (Lahiri & Bhowmik 1993; Sharma, 2017), the pathogen produces two types of infection i.e., local and systemic infection. Local infection is characterized by the formation of raised creamy white sporangial pustules on the under surface of leaves and on tender shoots whereas systemic infection is usually seen in young inflorescence and terminal leaves. Conventionally, fungicides are applied to reduce the disease incidence of white rust; however, improper and excessive use of chemically derived fungicides causes residual toxicity in the non-targeted organisms and leads to environmental degradation. Sustainable crop production needs eco-friendly methods of pest and diseases control. Therefore, the development and synthesis of bio-pesticides could be one of the options in conventional crop disease control system.

By nature, all plants synthesize and discharge numerous secondary metabolites, which enable them to defend against pathogens, pests, animal attacks and harsh environmental conditions (Cavoski, Caboni & Teodoro, 2011). According to Kumar, Singh, Sharma and Kishore (2017), *Ageratina adenophora* produces numerous secondary metabolites, which have antimicrobial (antibacterial and antifungal), antiseptic, analgesic, molluscicides and insecticidal potential. Although *A. adenophora* has meritorious chemical contents of diverse medicinal and antimicrobial properties, it is one of the most invasive weeds, where invasion of this weed has replaced larger part of the vegetation coverage and thus considered as a major threat to native biodiversity (Tripathi, Kushwaha & Yadav, 2006). Its allelopathic property makes it a noxious weed and dominates over other species (Subba & Kandel, 2013).In spite of potentially helpful biochemical characteristics and harmful biodynamic characteristic, the potential of *A. adenophora* for controlling fungal diseases in crops has not been evaluated (Sobrinho, de Morais, de Souza & dos santos Fontenelle, 2017). Thus, this study intended to evaluate the efficacy of fresh aqueous extracts of *A. adenophora* against White Rust of Crucifers.

2. Materials and Methods

2.1. Plant material collection, plant extracts preparation and media (PDA) preparation

Fully developed aerial parts of *A. adenophora* were collected from Sherubtse College campus, Kanglung, Trashigang, Bhutan. Aqueous extract of *A. adenophora* was prepared by grinding 20g of fresh leaf materials in an electric blender by adding sterile distilled water at the rate of 10 ml/g (Nashwa & Abo-Elyousr, 2012). The homogenates were filtered with Whatman No.1 filter paper. Then the filtrates were centrifuged at 5000 rpm for 10-15 minutes at room temperature and the supernatant were collected. The extracts were further diluted by adding sterile distilled water to have ranges of concentration (2.5%, 5.0%, 7.5% and 10%) and stored in refrigerator at 4°C. Potato Dextrose Agar medium (PDA) was prepared by dissolving 100g of potato infusion,

2.5g of dextrose and 10g of agar in 500ml of distilled water (pH 5.6±0.2). The dissolved medium was autoclaved at 15lbs at 121°C for 15 minutes.

2.2. Isolation of *Albugo candida*, antifungal activity, colony forming unit (CFU) and biomass evaluation

The infected plants were collected from local farmer in Kanglung gewog. The infected plant parts were cut, packed in the polythene bag and brought to the lab. They were thoroughly washed (leaf with shiny whitish pustules underside of *Brassica juncea*) in clean water followed by sterile water (distilled water), and with the help of sharp sterile razor blade, the infected tissues along with adjacent small unaffected tissue were cut into small pieces (25 mm squares). The cut pieces were transferred into sterile petri dishes containing 1% of sodium hypochlorite for surface sterilization for 30seconds. After surface sterilization, the sterilized pieces were transferred to petri dishes containing PDA and incubated at 25°C for 72 hours. A portion of mycelium from fungal colony was transferred to fresh potato dextrose broth for the pure culture.

The antifungal activity of aqueous extract of *A. adenophora* was assayed by poison-food technique and further confirmed by calculating Colony Forming Unit (CFU). The plant extract was incorporated into the molten PDB broth at a desired concentration at the ratio of4:1 (PDB and Plant extract) and then mixed thoroughly with Vortex Shaker. Then the medium was poured into 50 ml conical flask. The conical flasks were inoculated with 0.1 ml of fungal suspension and incubated in the incubator at 25°C for 48 hours. After 48 hours, 0.1 ml of fungal suspension serially diluted up to $10^4$was transferred and cultured on the petri plates containing PDA using spread plate method. The inoculated plates were incubated at 25°C for 48 hours. After 48 hours, the colonies on the petri dishes were counted under digital colony counter. The inhibitory activity of the extract was determined and evaluated using the following equation (1) modified from John, Ragi, Sujana & Kumar (2014):

$$IAG = \frac{NFC - NFT}{NFC} \times 100 \qquad (1)$$

Where, IAG = Inhibitory Activity of Growth, NFC = Number of Fungal colony in Control plate, NFT = Number of Fungal colony in Treated plates

To extract fungal biomass, 5 ml of different concentration (2.5%, 5%, 7.5% & 10%) of leaf extracts was incorporated into 20 ml potato dextrose agar broth in 50 ml conical flasks. The flasks were inoculated with 0.1ml of fungal inoculums. The cultures were incubated for 8 days at 25°C and the fungal biomass was harvested through centrifugation at 5000 rpm for five minutes. The fungal biomass pallets were collected and dried overnight in the oven at 35°C. The inhibitory activity of extract against fungal biomass was calculated using the following equation (2):

$$IAB = \frac{DWC - DWT}{DWC} \times 100 \quad (2)$$

Where, IAB = Inhibitory Activity on the Biomass, DWC = Dry Weight of biomass in Control, DWT = Dry Weight of biomass with extract Treatment

2.3. In vivo evaluation of extracts against *Albugo candida*

In vivo antifungal potential of *A. adenophora* aqueous extract was studied on potted plants under normal conditions. The potted plants were divided into Control (C), Curative Control (CC) and Preventive Control (PC). The plants in (C) and (CC) were infested with *A. candida* inoculum by spraying, when the symptoms appeared, the plants in CC were treated with the extract. For the preventive control test, the plants in PC were first treated with the extract by spraying and were infested with *A. candida* inoculum after 24 hours as per John et al., (2014).

2.4. Disease assessment

Table 1.Scoring method for evaluating the efficacy of *A. adenophora* extracts (Modified from Goss, Mafongoya, Gubba & Sam (2017))

Scale	Disease severity
0	No symptoms
1	Very few symptoms, 1-3 small lesions on one or two leaves
2	Small lesions on 3-5 leaves
3	Enlarged lesions on 3 or more leaves
4	Coalescing lesions forming wilted
5	Mildly chlorotic and appearance of green island as the leaf ages
6	Plants completely defoliated and dying

The disease severity index (DSI) was calculated by following equation (3) adopted from Alemu, Lemessa, Wakjira & Berecha (2014):

$$DSI = \sum \left[\frac{d \times n}{N \times m}\right] \times 100 \quad (3)$$

Where DSI = Disease Severity Index, d = disease rating on each plant, n = number of plants in each score, N = total number of plants examined and m = maximum disease rating possible.

The reduction of DSI on each plant was calculated using following equation (4):

$$PR = \left[\frac{PVC - PVT}{PVC}\right] \times 100 \quad (4)$$

Where, PR = Percent Reduction, PVC = Percentage Value of the Control and PVT = Percentage Value of the Treatment group.

3. Result and Discussion

3.1. Antifungal activity- inhibition percentage (IAF %) and Biomass of A. candida

Results revealed that all the extract concentrations (2.5%, 5%, 7.5% and 10%) showed positive results in suppressing the growth of *A. candida* with variable potency. The growth inhibition increased with increase in the extract concentration. The number of *A. candida* colony and biomass formation was found to be inversely proportional to the extract concentrations. The highest concentration (10%) of *A. adenophora* aqueous extract was found to be the most effective inhibiting *A. candida* growth in the lab. Therefore, 10% aqueous extract was chosen for experiments during the field test with the potted plants. Figure (1) and (2) depict the trends of inhibitory activity and biomass formation of *A. candida* in aqueous extracts of *A. adenophora*.

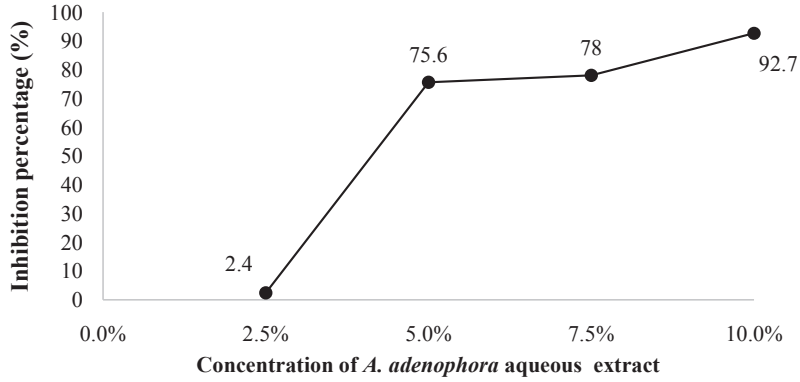

Figure 1.Inhibitory activity of aqueous extract of *A. adenophora* against *A. candida*

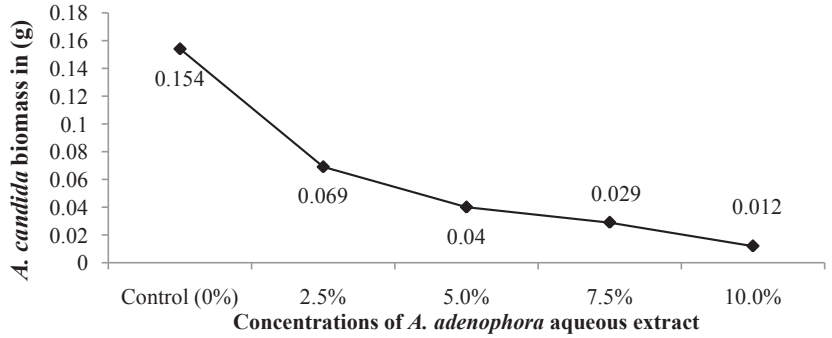

Figure 2.*A. candida* biomass formation in the presence of different concentrations of aqueous extracts *A. adenophora*.

The highest inhibitory activity of aqueous extract of *A. adenophora* against *A. candida* was observed in 10% concentration with 92.7% inhibition followed by 7.5% with 78% inhibition. The lowest inhibitory activity was observed in the lowest concentration (2.5%) with 2.4 percent

inhibition followed by 5% extract with 75.6% inhibition (Figure 1). There was a significant difference in the IAF of 2.5% and 5% of aqueous extract. The increase in IAF with extract concentration in the initial two concentrations was drastic. With one fold increase in extract concentration, inhibition increased by more than 30 folds from 2.4% to 75.6%. This may be due to increase in the metabolites concentration which is optimum for the inhibition. However, there was not much difference between 5% and 7.5%, possibly because the dead cells fenced the inhibitory chemicals to account for live cells. The other reason could be that the already dead cells might have absorbed most of the inhibitory chemicals from the extract leaving the probability of contact between live cells and inhibitory chemicals low (Zhang et al., 2013). Therefore, even with the increase in extract concentration to 10%, the increase in inhibitory effect is low (75.6% to 78%). Thus, IAF of aqueous extract of *A. adenophora* extract increased with the increase in the extract concentrations. On biomass front, the plate showed numerous colonies and massive growth of fungal mycelium in the control (0% extract), however, as the concentration increased, the fungal biomass and number of colony decreased (Fig 2). Thus, *A. adenophora* extract has a wide spectrum of fungistatic property against *A. candida.* The fungistatic activity of *A. adenophora* against *A. candida* may be due to the presence of secondary metabolites such as (mono-, sesqui-, di-, and tri-) terpenoids, phenylpropanoids, flavonoids, coumarins, sterols, alkaloids (Zhang et al., 2013), flavonoids, chromens, lactones, flavones and flavanones (Torres-Barajas et al., 2013). However, there was no attempt made to understand the phytochemicals of aqueous extracts of *A. adenophora* responsible for such an activity.

3.2. In vivo evaluation of aqueous extract of A. adenophora against A. candida - Disease Severity Index (DSI)

10% aqueous extract was selected for the *in vivo* test. The potted plants labelled as Control (C), Curative Control (CC) and Preventive Control (PC) was used for the test. The test plants (mustard green) infested with *A. candida* inoculums started to show symptoms after four weeks of infestation. The symptoms included distortion of young leaves and flowers, swelling on the stems and whitish lesions on the under surface of the leaves.

Table 2.Disease Severity Index (DSI) and % Reduction (PR) of disease incidence (*A. candida* vs aqueous extract of *A. adenophora*)

Weeks	Disease Severity Index (DSI)			% Reduction (PR) of Disease Incidence	
	C (%)	CC (%)	PC (%)	CC (%)	PC (%)
1	67.9	71.4	25.0		
2	78.6	75.0	32.1	3.6	54.1
3	80.1	77.2	36.8		

(C=Control, CC=Curative Control, PC=Preventive Control)

After symptom development on C and CC, the test plants were further observed for three weeks. In the first week, the highest DSI (71.4%) was recorded in CC test plants followed by control plants (67.9%). The lowest DSI was recorded in the PC test plants (25%). In the second and third week, the highest DSI was observed in the control. In the second week, the DSI were 78.6%, 75% and 32.1% for control, CC and PC respectively. Similarly, the DSI for the third week were 80.1% (Control), 77.2% (CC) and 36.8% (PC); from this it became evident that DSI recorded for three weeks is highest in CC plant as compared to the plants in PC. It is apparent that the infections are less severe in plants pre-treated with aqueous extracts of *A. adenophora* than those attempted to cure of infection. It is because the pre-treatment of the plants with aqueous extract of *A. adenophora* makes the environment around the plants unfavorable (toxic) for the infestation by *A. candida*. It might have also boosted the hosts' defense system by various phytochemicals.

3.3. % Reduction (PR) of disease incidence

The percent reduction was calculated using equation (4). PC was able to reduce infection by 51.4% whereas the CC was able to reduce infection by only 3.6% as compared to control. From this result, it is evident that the preventive control is much more effective than curative control to prevent the *A. candida* infection using aqueous extract of *A. adenophora*.

4. **Conclusion**

The study confirms that the aqueous extract of *A. adenophora* has excellent antifungal activity against *A. candida*. Thus, it demonstrates high potential in its use as alternative eco-friendly agent in controlling, reducing and managing *A. candida* infection and incidences in crucifers. Its antifungal activity is mainly due to the presence biochemicals such as alkaloids, flavonoids, chromens, flavones, diterpenes, sesquiterpenic, triterpenes and flavanones. However, a clear understanding of its bioactive phytochemicals vis-à-vis antimicrobial characteristic is essential so as to further affirm and validate its potential at the chemical and physiological level, and to use it as one of the important plant protection agents in integrated pest management (IPM) programs in subsistence organic farming. Secondly, it is doubly advantageous, in that by using it as an agent of plant disease control, the noxious weed population can also be effectively controlled.

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