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ANTIFUNGAL ACTIVITY OF LEAF EXTRACTS OF *POLYALTHIA LONGIFOLIA* (SONN.) BENTH. AND HOOK. F. AGAINST *RHIZOCTONIA SOLANI*

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ABSTRACT

Phytochemicals are chemical compounds which are also called as a secondary metabolite. Different types of secondary metabolites synthesized by plants which are play important role in biotechnological application and also in antimicrobial activity. In the present study were carried out to determine *in vitro* the antifungal activity of alcoholic, hydroalcoholic and aqueous crude extracts of *Polyalthia longifolia* leaf against *Rhizoctonia solani* fungus. All these extracts i.e., 100% aqueous, 100% alcohol and 50% hydro alcohol used as 10mg/ml concentration with acetone solvent to control of *Rhizoctonia solani*. The results revealed that the extract that most effectively inhibited growth was found to be 100% alcoholic leaf extract. Finding in this study confirmed that plant extracts can be used as to develop the plant extract-based bio-formulation to control plant pathogens to reduce the dependence on the synthetic fungicides which are ecological safe and cheap.

Keywords: Plant extract, Antifungal activity, Herbal-Bio formulation, Phytochemicals, Fungicides

INTRODUCTION

Plant pathogen fungi play negative effects on productivity of several economically important vegetable crop plants. Potato (*Solanum tuberosum* L.) is one of the most important food crops after wheat, maize and rice, contributing to food and nutritional security in the world. This crop is highly susceptible to black scruf diseases which caused by *Rhizoctonia solani.* This disease has been reported to cause marketable losses of up to 30% (Banville, 1989; Platt *et al.,* 1993). The total losses by this disease are reached up to 25% of the yield in western countries and almost 50% in developing countries (Bowyer, 1999) According to Sharma (2015) *Rhizoctonia solani* fungi causes yield loss up to 25% in the hill area, while in plain, it causes about 10% yield loss in potato crop. *Rhizoctonia solani* fungi forming sclerotia and mycelia in soil and also on plant debris part (Frank and Leach, 1980). The standard disease symptoms comprise decay of pre-emerging sprouts, blister on underground stem parts and stolen, reduced root systems and sclerotia formation on progeny tubers (typical black scurf symptoms) although, tuber-borne sclerotia of potato is formed so decline the quality of tuber (Jager *et al.,* 1991) with an alteration in number of tubers and target size and the development of deformed tubers (Anderson, 1982; Frank, 1978, 1981; Hide *et al.,* 1973; Carling *et al.*, 1989; Jeger *et al.,* 1996). In all over the world, scientists are associated in discovering methods or developing techniques to control of plant diseases. The most common and predominant method of disease control is chemical method because of its diverse use and ease of synthesis. The severe and rushed use of most of the synthetic fungicides has created various types of environmental and toxicological problems (Gurjar *et al.*, 2012). Therefore, present time focus is shifting towards the biological control of plant diseases and biological control programs, in order to gather key information on the long-term effects of the release (Myers and Bazely, 2003, Seastedt, 2015). To prevention plant diseases by formulation which is an inexpensive, ecologically safe fungicide made by combining plant extracts and organic materials. Synthetic fungicides are harmful for human that is the negative side of the use of synthetic fungicides. They have also harmful effect on ecosystem and soil fertility as well as wildlife health (Shiva *et al.* 2004). Hence in the present study effort will be made to develop the bio formulation using leaf extract of *Polyalthia longifolia* for systematic control of Black scruf disease of potato caused by *Rhizoctonia solani.*

MATERIALS AND METHODS

Test Pathogen

Collection of disease plant materials, isolation and purification of the pathogen

Infected tuber with sclerotia were collected from in the field near our house and also collected from vegetable market. First was to wash infected Tubers in tap water and then removing each sclerotia properly with help of a scalpel blade. Sclerotia surface was sterilized (1% NaOCl solution, 1 min), sterilized water used three times and rinsing the surface of Sclerotia. Then dried it well and placed on potato dextrose agar medium in Petri plates, which were incubated at 22°C for 4 days. Hyphae resembling *Rhizoctonia solani* were identified under a dissecting microscope, and hyphal tip isolation was carried out to establish pure cultures (Ogoshi, 1987). Hyphal tips were placed on potato dextrose agar (PDA) in Petri plates and incubated at 22°C for 7 days. Plates were then stored at 4°C for subsequent studies. The isolated fungus was identified under the microscope and we were also ordered *Rhizoctonia solani* by ICAR unit IARI at New Delhi.

Table no.1.1 Percentage extractive values of crude extracts of *Polyalthia longifolia* leaf

S. No.	Type of extract	Percent extractive value
1.	100% Aqueous	2.5
2.	100% Alcohol	4.2
3.	50% Hydro alcohol	4.6

Table no.1.2 Antifungal activity of crude extract of *Polyalthia longifolia* leaves against *Rhizoctonia solani*

S. No.	Type of extract	Growth Diameter after 7 days (mm)	%Mycelial growth inhibition
1.	100% Aqueous	31.33±0.57	61.15
2.	100% Alcohol	24.33±0.57	69.83
3.	50% Hydro alcohol	38.66±1.15	52.07
4.	Control	80.66±0.57	

Table no.1.3 stranded fungicide with water control (only PDA) against *Rhizoctonia solani*

S. No.	Standard fungicides and water control	Growth Diameter after 7 days (mm)	% Mycelial growth inhibition
1.	Mancozeb	21.33±0.57	73.55
2.	Bavistin	6.33±0.57	92.15
3.	Water (control)	80.66±0.57	-

Figure1.1.Isolation of test pathogen from infected tuber of potato and microscopic study of fungus

Preparation of crude extract

The fresh leaves of *Polalthia longifolia* plant were collected from Campus of University College of Science MLSU and the road side area of Udaipur. The collected plant material was dried on room temperature for 5 to 6 days until all its moisture out and become completely dry and then ground in an electrical grinder. The ground material was passed through a sieve of mesh size 60 to achieve a fine powder which was used to prepare the extract. Crude extract was prepared according to the cold extraction method (Shadomy and Ingraff, 1974). 100% aqueous, 50% hydro alcohol and absolute alcohol were used in Cold extraction method.20 gm dried powdered from plant leaf was suspended in 100 ml of respective solvent (50% hydro alcohol, Alcohol and water) for 48 hrs and then Whatman filter paper no.1 was used for filtered of the suspension then it dried through rotary vacuum evaporator.

Percent Extractive Value

The dried extracts were weighed and their percentage in term of the weight of dried plant material was determined by following formula:

$$\text{Percent extractive} = \frac{(\text{Weight of extract dried})}{(\text{Weight of dried material plant})} \times 100$$

Antifungal activity of crude extracts of *Polyalthia longifolia* (Sonn.) Benth. and Hook. f.

The inhibitory activity of crude alcoholic, 50% alcoholic and aqueous extract was done using poison food technique (Grover and Moore, 1962). In respect 100 mg of extract was dissolved in 10 ml acetone to prepare stock solution of 10mg/ml concentration. 1 ml of stock solution was mixed with 9 ml molten sterile PDA culture medium and further this mixture was poured into pre-sterilized petri-plates (9 cm diameters) and allowed to solidify at room temperature. Thus, prepared petri-plates were inoculated aseptically with 6mm disc of test pathogen's cultures. The petri-plates

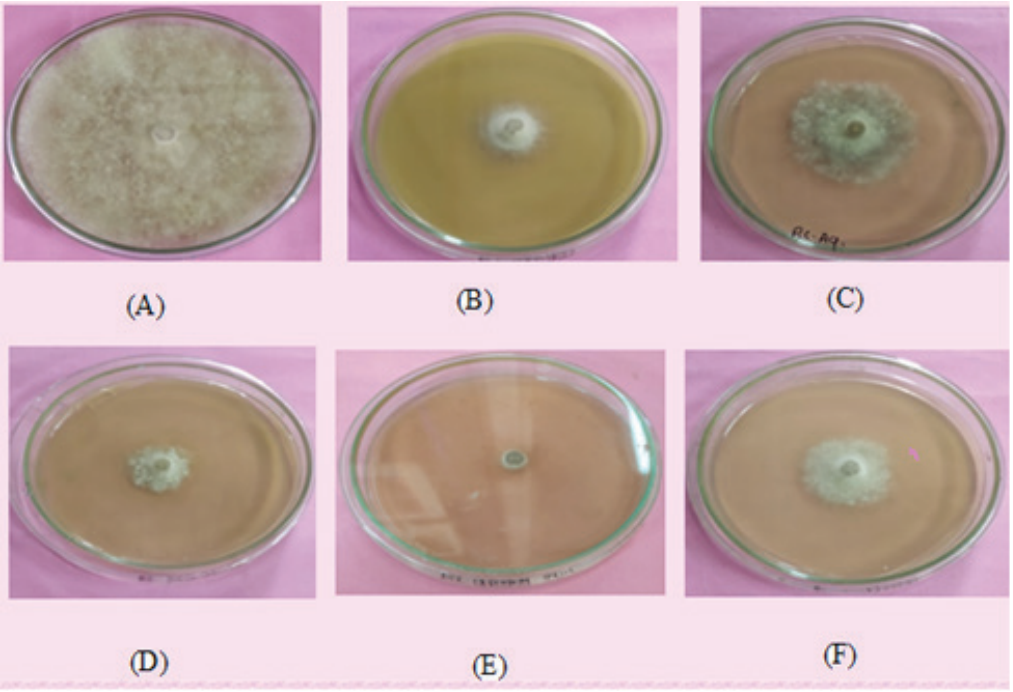

Figure 1.2 In-vitro antifungal of....

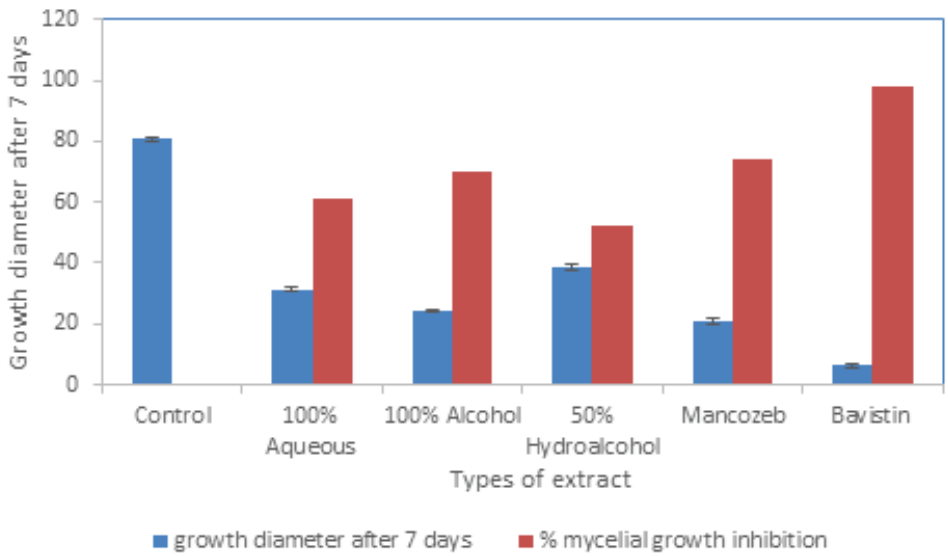

Figure: 1.3. Different type of crude extract of Polyalthia longifolia effect on growth diameter of Rhizoctonia solani fungus after 7 days showing in graph

were then incubated at 28±2C for seven days. Bavistin, mancozeb and only PDA culture media are used as control series along with test samples. Antifungal activity of extract was measured as a function of increasing in growth of 6 mm disc of inoculums.

After seven day of incubation the Average diameter of the fungal colonies was measured and mycelia growth in percentage was calculated by the following formula given below: (gc-gt)/gc×100

Mycelial growth inhibition:

gc= growth of mycelia colony after 7days incubation period in control set subtracting the diameter of inoculums disc.

gt= growth of mycelia colony after 7days incubation period in treatment set subtracting the diameter of inoculums disc.

RESULTS AND DISCUSSION

In the present study preparation of crude extracts and assay of antifungal activity of different type of crude extract were studied. The percentage extractive values of all extracts are depicted in table 1.1.The highest percentage extractive value was found in 50%Alcoholic extract followed by 100% Alcohol extract and Aqueous extract. The result of antifungal activity of crude extract of leaf of *Polyalthia longifolia* and stranded fungicide with water control (only PDA) are depicted in table no. 1.2 and 1.3 respectively. The best antifungal activity was observed with 100% alcoholic extract that the highest percentage of inhibition of mycelial founded in 100% alcoholic (69.83) followed by 100% aqueous (61.15) and 50% hydro alcohol (52.07).In the present study isolation of test pathogen from infected tuber of potato and preparation of crude extract and their antifungal activity were studies fig.1.1.

Different type of crude extract of *Polyalthia longifolia* effect on growth diameter of *Rhizoctonia solani* fungus after 7 days showing in graph Fig 1.3.

Discussion

Plants are important source of secondary metabolites i.e. alkaloids, flavonoids, phenolics, saponins, terpenes, lipids, carbohydrate etc (Cowan, 1999). These secondary metabolites play important role in controlling plant pathogens. There various techniques /methods are used to extraction of active ingredient from plant materials like leafs, seeds etc. The extraction of secondary metabolites can be done using cold or hot extraction method. Many researchers have been used cold or hot extraction method for successful expression of secondary metabolites (Yehia*etal*,2020;Grigoletto*etal*,2019;Boghsani*etal*., 2020;Chingwaru *et al*., 2020). Dileep *et al., (*2013) have been used aqueous extract of leaf and pericarp of *Polyalthia*

longifolia to control Fusarium oxysporum fungus in zingiber crop and *Pythium aphanidermatum.* Satish *et al.,* (2010) have been *study the* inhibitory effect of *Polyalthia longifolia* against sorghum grain moulds. In the present study we were used *Polyalthia longifolia* leaf extract to evaluate that antifungal activity against *Rhizoctonia solani which caused* Black scruf disease of potato. It is interesting to know that out of crude extract,100% alcoholic extract possesses highest inhibitory activity against *Rhizoctonia solani* These results hypothesized that plant extract of *Polyalthia longifolia leaf contain* active ingredient which inhibited the growth of test fungus. There are many researchers have investigated antimicrobial active compound which decrease the size of colony of fungus. Poison food technique is used commonly. This technique depends on the inhibition of microorganism's growth as an indication of sensitivity and is measured as a function of % inhibition. Several workers have used poison food technique for studying sensitivity of microorganism against plant extracts (Mohana *et al.*,2020;Chakrapani *et al.*,2020; Girish and Prabhavathi, 2019; Nene and Thapliyal, 2000). Antifungal effects of plant extracts can be due to existence of various phytochemicals that can act alone or in synergy to inactive or kill the microorganism. It has been proved that there is a important relationship between extract and active compounds. (Callixte *et al*., 2020; Sheekh *et al*., 2020).There are several methods which are used to evaluate antifungal activity of plant extract (kohli *et al*., 2002).In support to this hypothesis many researchers found *Polyalthia longifolia* has Pharmacological activities, namely anti-inflammatory, anti-arthritic, antiulcer, anticancer, antioxidant, antidiabetic and analgesic activities has been reported in *Polyalthia* plant (Cibin *et al.*, 2012; Gupta *et al.*, 2014; Nag *et al.*, 2015; Ahmad *et al.*, 2016).). *P. longifolia* possess antiulcer activities (Malairajan *et al.*, 2008), hepato protective anti-inflammatory (Tanna *et al.*, 2009) activities, hypoglycemic and anti hyperglycemic (Nair *et al.*, 2007) activities, and antimicrobial activity (Faizi *et al.*, 2003; Murthy *et al.*, 2005; Nair and Chanda, 2006). So we can say that *Polyalthia longifolia* is very important plant. The extract of leaves of *Polyalthia longifolia* shows significant antifungal activity. The result indicate that this plant *Polyalthia longifolia* can be used for developing the plant extract based Bio-formulation for effective control of Black scruf disease of potato in eco-friendly way.

CONCLUSIONS

The results of the present studies would suggest that use of herbal formulation from leaf of *Polylathia longifolia* control of Black scruf disease of potato compared to fungicides which are costly and hazardous and can also be used to develop Bio-formulation to control of Black scruf disease of potato in the way of eco-friendly.

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