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# Study of Various Fungitoxic Properties of Aqueous Extract of *Hedychium spicatum* against *Rhizoctonia solani* Kuhn.

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**The aqueous extract of *Hedychium spicatum* was screened for its antifungal activity against *Rhizoctonia solani* Kuhn. a causal organism of Black scurf disease of potato. The extract of *Hedychium spicatum* showed strong antifungal activity against *Rhizoctonia solani*. The present study highlights the fungitoxic properties of aqueous extract of *Hedychium spicatum*. The Minimum Inhibitory Concentration (MIC) of *Hedychium spicatum* was found to be 0.5 ml. The nature of toxicity of extract was fungicidal at its MIC. The fungitoxicity of the extract was thermostable up to 60°C.The extract was found to be active even after the storage of 360 days and had no effect on its fungitoxicity. On increasing the number and diameter of fungal discs of the test fungus there was no effect on fungitoxicity, indicating their capacity to sustain heavy inoculums at MIC. The extract of *Hedychium spicatum* was found to exhibit a broad range of fungitoxic spectrum by inhibiting the mycelial growth of 20 different fungi at its MIC. The fungitoxicity of extract was found greater when compared with some prevalent synthetic chemical fungicides. Therefore, the aqueous extract of *Hedychium spicatum* can be recommended as potential source of ecofriendly herbal fungicide.**

**Keywords:** Aqueous extract, Fungitoxic properties, *Rhizoctonia solani* Kuhn., *Hedychium spicatum*.

Chemical fungicides are very costly and cause serious problems. They reduce the quality of crops, have toxic effect on non-target organisms cause environmental pollution and resistance in pests and disease agents (Kagal *et al*., 2004).

The plant world is a rich store house of natural chemicals that could be exploited for use as natural fungicides (Satish *et al.,* 2007). Therefore the control strategies are now directed towards replacing the use of hazardous chemical fungicides by environmentally friendly natural products (Mamdouh & Eweis, 2007). Higher plants are known to express toxicity against spore germination and mycelial growth of phytopathogenic fungi (Samuel *et al*. 1995). The natural products, extracted from plants are now proved to be safer, ecofriendly, non pollutive and effective to control phytopathogens.

Fungal diseases cause considerable loss in many economic crops. Black scurf disease of potato caused by the fungus *Rhizoctonia solani* Kuhn. is a serious disease of potato in India. It causes 10–25% yield loss in India depending upon growing regions besides this it also decreases the market acceptability due to presence of black sclerotia on surface of the tubers. The *Rhizoctonia solani* may also attack and kill emerging sprouts in soil and results in reduced crop stand and thus cause more loss in yield.

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Black scurf is distributed in India in different regions at different levels of severity and is a major disease problem in fields where potato is cultivated year after year in the same field (Khurana *et al.,* 1998; Arora, 2012).

In recent years there has been a gradual revival of interest in the use of medicinal plants because herbal medicines have been reported to be safe and without any adverse side effects. Recent researches reveled that some products of plants origin have been investigated to be an effective source of strong fungicidal activity without undesirable side effects.

The extract of *Hedychium spicatum* (Chaudhary *et al*. 2017), having strong antifungal activity against the *Rhizoctonia solani*. Therefore the present work was designed to explore the fungitoxic properties of aqueous extract of *Hedychium spicatum* against *Rhizoctonia solani.*

## MATERIALS AND METHODS

### Plant materials

In the present study, rhizome of *Hedychium spicatum* is collected from different areas of Gorakhpur, to analyze their fungitoxic property against *Rhizoctonia solani* Kuhn. This work was done in the year 2015 and 2017.

### Preparation of extract

### Aqueous Extract

For the preparation of aqueous extracts, 10g of each dried sample was grinded into a fine powder with 100 ml sterile distilled water and left for overnight at room temperature (30 ± 2°C). The content of the flask was then filtered through filter paper to obtain clear infusion in laminar air flow (Chaudhary and Tariq, 2006). Poisoned food technique was used for the evaluation of fungicidal potential (New, 1971).

### Microbial Cultures and Growth Conditions

The plant extracts were assayed for fungicidal activity against the fungal strain *Rhizoctonia solani* Kuhn. (MTCC No. 4633) obtained from Microbial Type Culture (MTCC), Chandigarh. This fungus was grown on PDA plate at 27°C ± 2°C and maintained with periodic sub – culturing at 4°C.

### *In vitro* investigation of fungitoxic properties of aqueous extract of *Hedychium spicatum* against *Rhizoctonia solani* Kuhn

The study of fungitoxicity was done by Poisoned Food Technique (New, 1971). For treatment sets, 1 ml of the prepared extract was mixed with 9 ml of molten PDA medium in a pre-sterilized Petri plate and the contents were agitated in a circular mode in order to mix the extract homogeneously. Media without plant extract served as control. Chloramphenicol was added to the medium to prevent bacterial growth (Francisco et al. 2010).

A fungal disc (5 mm in diameter) cut from the periphery of 7 days old culture of *Rhizoctonia solani* Kuhn. with the help of flame sterilized cork borer, served as inoculums was placed at the centre of each Petri plate. The plates were incubated for 7 days at 27 ± 2°C. The experiment was performed under aseptic lamina conditions and replicated three times.

Colony diameters in mutual perpendicular directions were measured on the seventh day in assay plates. Fungitoxicity was recorded in terms of the % inhibition of mycelial growth and calculated using the following formula (Singh and Tripathi, 1999).

$$\text{Percent inhibition of mycelial growth} = \frac{dc - dt}{dc} \times 100$$

Where

*dc* – average diameter of fungal colony in control sets.

*dt* – average diameter of fungal colony in treatment sets.

### Minimum Inhibitory Concentration (MIC)

The MIC of the extract required for absolute inhibition of mycelial growth of the test fungus, *Rhizoctonia solani* Kuhn., was determined by the Poisoned Food Technique (New, 1971). Requisite amounts of the prepared extract were added to pre-sterilize Petri plates containing 10 ml of molten PDA medium. Now the different concentration of the extract is added to the medium. The contents of the plates were agitated in a circular mode to mix the extract in the medium evenly. In control sets, the same amount of sterilized distilled water was used in place of the extract. The assay plates were incubated for six days at 27 ± 2°C. The observations were recorded on the seventh day in terms of the percent inhibition of mycelial growth and data presented in Table -1 are based on the averages of all the replication.

**Effect of temperature**

One ml of extract was taken in glass vials which is closed tightly and exposed to the desired temperature in electrical oven for an hour. The vials were then allowed to cool down to room temperature and fungitoxicity of extract was assessed (Table - 2).

**Effect of storage**

Fresh extract was stored in airtight glass vials at room temperature. The antifungal activity of the stored extract was tested at intervals of 30 days (Table 3).

**Effect of Increased Incoulum**

The effect of increased inoculum was studied in terms of increased number of inoculum discs as well as increased diameter of inoculum discs.

The PDA medium was autoclaved and poured into pre-sterilized Petri plates. Requisite amount of extract was mixed with PDA medium and allowed to solidify. After complete solidification of the medium, Petri plates were inoculated with increasing the number of disc and incubated at. 27 ± 2°C Observation was recorded on seventh day (Table - 4).

In the similar way the PDA medium was prepared. After complete solidification of the medium, Petri plates were inoculated with increasing the diameter of discs and incubated at 27 ± 2°C. Observation was recorded on seventh day for presence (+) and absence (-) of growth of the test fungi (Table - 5).

**Range of antifungal spectrum of extract**

The range of antifungal activity of the aqueous extract at 0.5 ml dilution was tested against twenty fungal pathogens, viz. *Aspergillus flavus* Link., *Aspergillus fumigatus* Fres., *Aspergillus nidulans* (Eidam) Winter, *Aspergillus niger* van Tiegh., *Aspergillus ochraceus* Wilhelm, *Aspergillus ruber* Thom & Church, *Aspergillus terreus* Thom, *Botryodiplodia theobromae* Patouillard, *Chaetomium indicum* Corda, *Cladosporium herbarum* (Pers.) Link., *Curvularia geniculata* (Tracy & Earle) Boedijn, *Curvularia lunata* (Wakker) Boedijn, *Drechslera hawaiiensis* (Bugn.) Subram. & Jain., *Drechslera sativa, Fusarium acuminatum* Ellis & Everhart, *Fusarium oxysporum* schlecht., *Macrophomina phaseoli* (Maublanc) Ashby, *Nigrospora oryzae* (Berk. & Broome) Petch, *Sclerotium oryzae* Catt., *Sclerotium rolfsii* Saecardo using the Poisoned Food Technique as described previously. Experiments were repeated twice and each set contained three replications. The results recorded in terms of the % inhibition of mycelial growth based on the mean values of all replications are given in Table - 6.

**Table 1.** Minimum Inhibitory Concentration (MIC) of aqueous extract of*Hedychium spicatum*

| Concentration of extract (ml) | % inhibition of mycelial growth of *Rhizoctonia solani* Kuhn |
|---|---|
| 1.0 | 100 |
| 0.9 | 100 |
| 0.8 | 100 |
| 0.7 | 100 |
| 0.6 | 100 |
| 0.5 | 100 |
| 0.4 | 90 |
| 0.3 | 67 |
| 0.2 | 44 |
| 0.1 | 21 |

**Table 2.** Effect of temperature on fungitoxicity of extract

| Name of the Plant | Temp. °C | % inhibition of mycelial growth of *Rhizoctonia solani* Kuhn. | |
|---|---|---|---|
| | | Control | Treatment |
| *Hedychium spicatum* | 30 | 0 | 100 |
| | 40 | 0 | 100 |
| | 50 | 0 | 100 |
| | 60 | 0 | 100 |
| | 70 | 0 | 80 |

**Table 3.** Effect of storage on fungitoxicity of extract

| Storage period (Days) | % inhibition of mycelial growth of *Rhizoctonia solani* Kuhn |
|---|---|
| 30 | 100 |
| 60 | 100 |
| 90 | 100 |
| 120 | 100 |
| 150 | 100 |
| 180 | 100 |
| 210 | 100 |
| 240 | 100 |
| 270 | 100 |
| 300 | 100 |
| 330 | 100 |
| 360 | 100 |

**Table 5.** Effect of increased inoculum on fungitoxicity of extract (By increasing the diameter of discs)

| Size of disc inoculated ( diameter in mm) | mycelial growth of | |
|---|---|---|
| | Control | Treatment |
| 5 | + | - |
| 6 | + | - |
| 7 | + | - |
| 8 | + | - |
| 9 | + | - |
| 10 | + | - |
| 12 | + | - |

(+) : presence of mycelial growth
(-) : absence of mycelial growth

## RESULTS

In the present study, the MIC of the aqueous extract of *Hedychium spicatum* was found 0.5 ml i.e. the aqueous extract completely inhibited the mycelial growth of the test fungus at 0.5ml dose indicating its Minimum Fungicidal Concentration (MFC) (Table - 1).

The study reveals that the extract retained its fungitoxicity when it was exposed up to $60^0$C temperature, however when it was exposed above than $60^0$C temperature there was significant loss in their fungitoxic potential (Table -2).

The extract was stored up to 360 days in separate airtight glass vials at room temperature.

**Table 4.** Effect of increased inoculum on fungitoxicity of extract (By increasing the number of discs)

| No. of disc inoculated (5mm diameters) | Mycelial growth of *Rhizoctonia solani* Kuhn. | |
|---|---|---|
| | Control | Treatment |
| *2* | + | - |
| 4 | + | - |
| 6 | + | - |
| 8 | + | - |
| 10 | + | - |
| 12 | + | - |
| 14 | + | - |

(+) : presence of mycelial growth
(-): absence of mycelial growth

The antifungal activity of stored extract was tested at its respective MIC at the interval of 30 days. The result recorded in (Table-3), revealed that fungitoxicity of extract even after storage up to 360 days at room temperature, remained unchanged. This clearly indicates that the storage has no effect on the fungitoxicity of the aqueous extract of *Hedychium spicatum*.

The inoculum of test fungus was increased in terms of number and size of discs per Petri plate. The result recorded in (Table - 4) showed that extract was able to inhibit all the 12 discs, each of 5mm diameter and on increasing the diameter (Table -5) of the test fungi indicating their capacity to sustain heavy inoculum at their respective MIC.

The results presented in (Table – 6) revealed that the aqueous extract of *Hedychium spicatum* at 0.5 ml dose completely inhibited the mycelial growth of *Aspergillus flavus* Link., *Aspergillus nidulans* (Eidam) Winter, *Aspergillus niger* van Tiegh., *Aspergillus terreus* Thom, *Cladosporium herbarum* (Pers.) Link., *Fusarium oxysporum* schlecht., *Macrophomina phaseoli* (Maublanc) Ashby, *Sclerotium oryzae* Catt., *Sclerotium rolfsii* Saecardo. However, it also showed moderate toxicity to other fungi tested.

## DISCUSSION

Swaminathan (1978) found that green plants are reservoir of biotoxicants and inexhaustible source of number of pesticides.

**Table 6.** Range of antifungal spectrum of extract

| S. No. | Pathogen | % of inhibition on mycelial growth at 0.5 ml concentration |
|---|---|---|
| 1 | *Aspergillus flavus* Link. | 100 |
| 2 | *Aspergillus fumigatus* Fres. | 62 |
| 3 | *Aspergillus nidulans* (Eidam) Winter | 100 |
| 4 | *Aspergillus niger* van Tiegh. | 100 |
| 5 | *Aspergillus ochraceus* Wilhelm | 82.5 |
| 6 | *Aspergillus ruber* Thom & Church | 90 |
| 7 | *Aspergillus terreus* Thom | 100 |
| 8 | *Botryodiplodia theobromae* Patouillard | 72.3 |
| 9 | *Chaetomium indicum* Corda | 70 |
| 10 | *Cladosporium herbarum* (Pers.) Link. | 100 |
| 11 | *Curvularia geniculata* (Tracy & Earle) Boedijn | 85 |
| 12 | *Curvularia lunata* (Wakker) Boedijn | 78 |
| 13 | *Drechslera hawaiiensis* (Bugn.) Subram. & Jain. | 76.5 |
| 14 | *Drechslera sativa* | 100 |
| 15 | *Fusarium acuminatum* Ellis & Everhart | 73.6 |
| 16 | *Fusarium oxysporum* schlecht. | 100 |
| 17 | *Macrophomina phaseoli* (Maublanc) Ashby | 100 |
| 18 | *Nigrospora oryzae* (Berk. & Broome) Petch | 67.8 |
| 19 | *Sclerotium oryzae* Catt. | 100 |
| 20 | *Sclerotium rolfsii* Saecardo | 100 |

Kumar *et al.,* (1995) found that in comparison to synthetic compound, the pesticidal compounds of plant origin are more effective and have little or no side effects on human beings. Similarly, Hooda and Srivastava (1998) have mentioned that natural fungicides are free from environmental toxicity as compared to synthetic compound, natural compounds are less phytotoxic, easily biodegradable (Saxena *et al.,* 2005). The extensive use of agrochemicals especially fungicides, which leads more carcinogenic risk than other pesticides which may give rise to undesirable biological effects on animals and human beings (Osman and Abdulrahman, 2003).

The present study reveals a report on the fungitoxic properties of extract of *Hedychium spicatum*. Determination of the Minimum Inhibitory Concentration (MIC) of a fungitoxicant is necessary in order to know its appropriate dose for the complete mycelial inhibition of the test pathogen. High doses of a fungitoxicant may lead to a decrease in its rational value, increase its wastage and may also considerably deteriorate the quality of the crop treated. Although a large number of plants and their products have been tested for their antifungal activity, only a few have been studied in detail with respect to their MIC. The study showed that the extract of *Hedychium spicatum* was fungicidal at its MIC.

According to Wellman (1977) the antifungal efficacy of a fungitoxicant must not be affected by extremes of temperature. Arnold (1958) recorded loss of antifungal activity in *Oenthera argentina* and *O. odorata*, when the plants were treated beyond $60^0$C. Bilbruck (1958) and Nene and Thapliyal (1965) reported that temperature had no effect on the antifungal activity of the plants. Pandey (1983) reported that the antifungal activity of *Chenopodium album* and *Lantana camara* remained unaffected upto $100^0$C. Magdy *et al.* (2014) also reported that the activity of *Cinnamomum cassia, Alium sativum, Syzygium aromaticum, Punica granatum, Citrus lemoniumn* and *Hibiscus sabdariffa* plant extracts were not affected when exposed to different temperatures ranging from 4 °C, 30 °C, 60 °C and 90 °C. The present study reveals that the fungi toxic properties of the extract of *Hedychium spicatum* do not alters even if the extract is exposed to high temperature i.e. upto $60^0$C however when it was exposed above

than $60^0$C temperature there was significant loss in its fungitoxic potential. The temperature resistance studies indicate that the phytoconstituents are thermostable, but heating beyond 60°C leads decrease in the antifungal activity, this may be due to volatilization of components or due to some physical and chemical changes in molecules of natural products during heating.

The biological property of an extract may change upon storage due to chemical modification of its various components (Chatwal, 1983). Arnold (1958) observed that *Oenthera argentenea* retained its activity at least 40 days on storage. The antifungal activity of heart wood of *Abies balsamea* remained unaffected when it was stored at room temperature for 3 years (Etheridge, 1962). Misra (1975) observed that extract of *Clematis gouriana* and *Ranunculus scleratus* remained active upto 15 days of storage. In the present study it was found that the toxicity of extract was not affected by storage upto 360 days indicating that presence of durable active principles.

The efficacy of antifungal substances depends upon the density of the organism it has to combat (Skinner, 1955). Mishra (1975) reported that fungitoxicity of *Clematis gouriana* and *Ranunculus scleratus* decreased on account of increase in the inoculums. In the present investigation it has been observed that the extract was able to inhibit all the 12 discs, each of 5mm diameter (Table-4) and on increasing the diameter (Table -5) of the test fungi indicating their capacity to sustain heavy inoculum at their respective MIC. This is the important potential of the extract to be exploited as natural fungicide. Inhibiting the fungal growths against number and increased inoculums reveals strong fungitoxic property.

A fungitoxicant may exhibit broad range of spectrum inhibiting many fungi or may be effective against some specific ones. If a fungitoxicant possesses a narrow range of toxicity, it cannot be successfully employed in controlling the disease incited by a complex of pathogens (Christensen, 1972; Kulik, 1973). Such fungitoxicants may be potentially valuable in controlling few diseases but their limited market value would make them ineffective for producers. Fungitoxicants with broad spectrum offer greater promise because of potentially larger market value. In the present investigation the extract of *Hedychium spicatum* exhibited a broad range of the antifungal spectrum.

## CONCLUSION

The preliminary *in vitro* investigations revealed that the aqueous extract of *Hedychium spicatum* has strong fungicidal efficacy at low MIC against *Rhizoctonia solani*, inhibiting heavy doses of inoculums, long shelf life, thermostable and having broad antifungal spectrum. All these observations and findings suggest that the aqueous extract of *Hedychium spicatum* has the potential of becoming powerful and safe alternative means of disease control agent than the harmful chemical fungicides.

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