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Phytotoxic evaluation of plant extracts on seed germination & seedling growth of *Solanum tuberosum* and fungitoxic efficacy of extracts in soil condition

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Abstract
The present studies cover the phytotoxic effect of two plant extracts viz. *Curcuma malabarica*, and *Hedychium spicatum* on *Solanum tuberosum* with respect to seed (tuber) germination and seedling growth (shoot length) and fungitoxic efficacy of extracts in soil condition**.**
The seeds (tubers) of *Solanum tuberosum* exposed to selected plant extracts at 5.0 ml dose and the phytotoxicity of extracts was measured in terms of per cent seed germination and the seedling growth in terms of plumule length. The results showed that the germination of seeds (tubers) and the seedling growth of *Solanum tuberosum* remained unaffected.
The study also revealed that the fungitoxic efficacy of extracts in soil condition after re-inoculation of test fungus, remained unchanged.

Keywords: phytotoxic effect, plant extract, soil condition, antifungal activity

Introduction
The growing world population demands for a stable crop production, which requires controlling of plant diseases (Strange & Scott, 2005) [25]. Agriculture is the backbone of economy and majority of population depends upon agriculture especially potato crop. As a consequence, management strategies including the use of chemical fungicides are often employed inappropriately and indiscriminately. Furthermore, fungi are continually becoming resistant to fungicides and they are at risk of being withdrawn from the market (Arcury and Quandt, 2003; Deising *et al*, 2008) [5]. In addition to reducing crop yield, fungal pathogens often degrade the quality of crop by producing toxins that affect human health. Thus, the replacement of synthetic fungicides by natural products that are nontoxic and specific in their action is gaining considerable attention (Costa *et al.* 2000, Duru *et al.* 2003, Sanjay, 2009) [7, 9, 19].
Higher plants and their products are known to possess fungitoxicity against spore germination and mycelial growth of phytopathogenic fungi (Varma and Dubey, 1999) [22]. The plant world is a rich store house of natural chemicals that could be exploited for use as fungicides (Satish *et al.*, 2008) [20].
Plants have been known for their fungitoxic properties since ancient times. Approximately 2400 plant species are known to possess biologically active compounds that control various pathogens effectively. Conspicuously there are more than 10,000 secondary metabolites that have been found to have a role in plant defense out of 400,000 plant chemicals (Hamburger and Hostettmann, 1991) [13]. The antifungal properties of different plant extracts against plant disease have also been observed by several researchers (Mishra and Tewari, 1990; Ali *et al*., 1992; Akhtar *et al*., 1997; Suberu, 2004) [15, 3, 1, 26].
The higher plants contain a wide spectrum of secondary metabolites having antimicrobial activity. Plant biochemicals and crude extracts have also been reported to have antimicrobial properties against plant pathogens *in vitro* as well as *in vivo* and are used as bio-fungicidal compounds (Fawzi *et al*., 2009; Al-Askar and Rashad, 2010) [11, 2]. Further, the use of botanicals is regarded as the best suited ecofriendly measure as they are easily biodegradable and safer.
Some plant extracts may have aggressive properties by producing the phytotoxic or plant growth inhibiting substances that adversely affect growth and development of plants. Several chemicals can be released together and may exert toxicities in an additive or synergistic manner (Putnam and Tang, 1986) [18]. However, there are several reports indicating that most of the chemicals at high concentrations are phytotoxic and show detrimental effects on plant growth and seed germination although some chemicals are also identified as fungicidal in nature.
Therefore, the aim of the present investigation is to study the phytotoxic effect of two plant extracts viz. *Curcuma malabarica* and *Hedychium spicatum*on with respect to seed (tuber) germination and seedling growth (shoot length) and fungitoxic efficacy of extracts in soil condition in order to evaluate the potential of these extracts as a valuable resource of natural fungicides or as a plant growth regulator.

Materials and Methods
Plant Material
Fresh plant species were collected from Gorakhpur district, Uttar Pradesh in sterile polythene bags. The collected plant materials were identified with the help of floras and the identification was confirmed with authentic herbarium specimens logged in the herbarium of the department. The plant materials were surface sterilized using 2% sodium hypochlorite and rinsed twice with sterile distilled water before use.

Preparation of Extracts
Crude extract: 20 grams of freshly collected disease-free leaves and rhizome of plants were surface sterilized with

sodium hypochlorite solution (4%) for 2 min followed by washing with sterilized distilled water to remove all the traces of sodium hypochlorite. The sample was then chopped into small pieces and macerated to pulp using a sterilized pestle and mortar. The pulp was squeezed by double layered sterilized muslin cloth and filtered through Wattman's No. 1 filter paper. The crude extract thus obtained was subjected to antifungal testing against the test fungus.

Aqueous Extract: For the preparation of aqueous extracts, 10g of each dried sample was ground into a fine powder with 100 ml sterile distilled water and left for overnight (24 hours) 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 antifungal potential (New, 1971) [17].

Fungal 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 27^0C ± 2°C and maintained with periodic sub – culturing at 4^oC. *Rhizoctonia solani* causes Black Scurf disease in potato plants.

Determination of Phytotoxicity

The phytotoxicity of selected plant extracts viz. *Curcuma malabarica* and *Hedychium spicatum* were studied with respect to seed (tuber) germination and seedling growth (shoot length).

Effect on Seed (tuber) Germination

Extract of fresh plant was prepared as described earlier. For treatment sets, surface sterilized seed of *Solanum tuberosum* were soaked in 5 ml of extract for 5 hours. Same amount of seeds (tubers) were soaked in sterilized water for the same period for control sets. Treated as well as control seed were separately placed on three layers of moist filter paper for 6 days in petriplates, which were incubated at room temperature. Observations were recorded in terms of the percent seed (tuber) germination. Each set contained three replicates.

Effect on Seedling Growth (shoot length)

The germinated seeds (tubers) of previous sets were allowed to grow upto eight days and the length of plumule of each germinated seed in control and treatment sets were recorded.

$$\% \text{ inhibition of shoot length} = \frac{\text{Plumule length in test sample}}{\text{Plumule length in in control}} \text{ X } 10$$

Fungitoxic Efficacy of Extracts in Soil Condition

Experiments were conducted in the presterilized culture tubes having a hole at the bottom. The hole of the tubes was plugged with sterilized cotton. A small amount of presterilized soil was put in the tubes. The tubes were then inoculated aseptically with a 5 mm disc of test fungus, obtained from the periphery of 7 days old culture. The disc in the tubes was again covered with sterilized soil. In treatment sets plant extracts were poured in sufficient amount so as to reach upto the bottom of the tubes. Similarly, sterile water was poured in control sets.

All the tubes were kept at room temperature for 6 days. After 6 days of incubation, fungal disc was taken out and inoculated in petriplates containing PDA medium. The plates were observed upto 7 days for the revival of growth of the fungus.

Results

Phytotoxicity and determination of fungitoxic efficacy in soil condition are important attributes in determination of fungitoxic potential of plant extracts. Determination of phytotoxicity of a plant species help in the formulation of natural plant growth regulators or natural fungicides. During the present investigation the crude and aqueous extracts of two plant extracts viz. *Curcuma malabarica*, and *Hedychium spicatum* were studied with respect to seed (tuber) germination & seedling growth (shoot length) and fungitoxic efficacy of extracts in soil condition.

The phytotoxicity of extracts was measured in terms of percent seed germination and the seedling growth in terms of plumule length of *Solanum tuberosum*.

The result recorded in Table -1 showed that the germination of seeds (tubers) of *Solanum tuberosum* remain unaffected when seeds were soaked in the extracts of *Curcuma malabarica* and *Hedychium spicatum.*

The seeds of *Solanum tuberosum* after germination were allowed to grow for eight days. The observations were recorded in Table-2 and Fig. -1 revealed that there was no adverse effect on the plumule length of treatment sets. It is, therefore, apparent from above observation that the extract of *Curcuma malabarica* and *Hedychium spicatum* did not have any phytotoxicity on *Solanum tuberosum*.

The antifungal activity of extracts in soil conditions recorded in Table-3 revealed that after re-inoculation there was no growth in fungal disc upto 7 days. This indicates that the extracts remain fungitoxic, when placed in soil.

Table 1

Effect of selected plant extracts on tuber germination of *Solanum Tuberosum*			
Germination Period (Days)	**% Tuber Germination**		
	Control	***Curcuma malabarica***	***Hedychium spicatum***
2	100	100	100
3	100	100	100
4	100	100	100
5	100	100	100
6	100	100	100

Table 2

Effect of selected plant extracts on shoot length of *Solanum tuberosum*				
Age of Seedlings (Days)	**Plumule length of seedling (cm)**			
	Curcuma malabarica		***Hedychium spicatum***	
	Control	**Treatment**	**Control**	**Treatment**
3	3.2	3.3	3.2	3.4
4	4.6	4.7	4.6	4.8
5	5.3	5.5	5.3	5.6
6	6.1	6.2	6.3	6.5
7	6.5	6.4	6.6	6.8
8	6.9	7.0	7.0	7.1

Table 3

Fungitoxic Efficacy of Selected Plant Exracts in Soil Condition				
Number of Days	Mycelial Growth of Fungal Disc (*Rhizoctonia solani* Kuhn.) in Soil			
	Curcuma malabarica		*Hedychium spicatum*	
	Control	Treatment	Control	Treatment
3	+	-	+	-
4	+	-	+	-
5	+	-	+	-
6	+	-	+	-
7	+	-	+	-
8	+	-	+	-
(+): presence of mycelial growth (-): absence of mycelial growth				

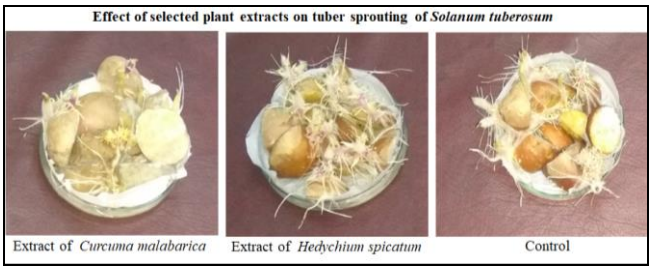
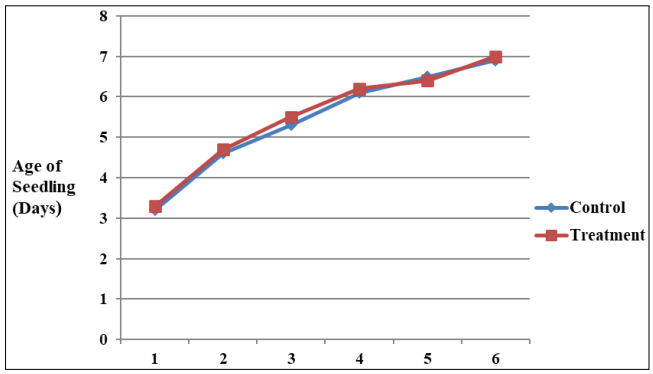
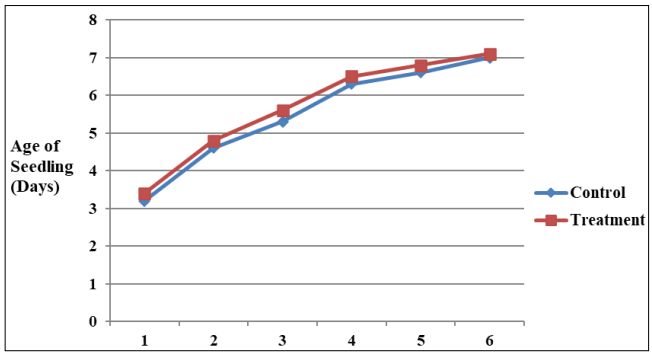


Plate 1



Graph 1: Effect of *Curcuma malabarica* plant extract on shoot length of *Solanum tuberosum*



Graph 2: Effect of *Hedychium Spicatum* extract on shoot length *Solanum tuberosum*

Discussion

From agricultural point of view the phytotoxic potential of plants origin is very beneficial and helpful to screen substances possessing fungicidal effect (Khuda F *et al.*, 2012) [14]. Wellman (1977) [29] emphasized that any compound possessing fungitoxicity must be evaluated for its phytotoxicity before subjecting it to *in vivo* trials. Therefore, it was decided to test the phytotoxicity of plant extracts exhibiting fungitoxicity. Previously, the extracts of *Allamanda cathartica, Lawsonia inermis* and *Eucalyptus citriodora, Ruellia tuberosa, Ricinus communis* were found to be non-phytotoxic by Tripathi *et al.* (1978) [8], Dixit *et al.* (1982) [27], and Sharma (2008) [21], respectively. However, Fawcett *et al.* (1969) [10] and Staron *et al.* (1969) [24] reported that 'wyerone' from *Vicia faba* and 'anagalloside' from *Anagallis arvensis* were phytotoxic. The results shown in Table-1 revealed that soaking seed of *Solanum tuberosum* in the extract of *Curcuma malabarica,* and *Hedychium spicatum* for different time periods has no significant effect on its germination. This indicates that the extracts have no phytotoxic effects as far as the seed germination of *Solanum tuberosum* is concerned.

Some publications clearly indicate the efficacy of plant crudes and their products in the control of various diseases in field conditions (Mosch & Zeller 1989; Alice & Sivaprakasam 1996) [16, 4]. The control of soil-borne pathogens under *in vivo* conditions has generally been attempted by seed treatment or by soil amendment. Some researchers successfully cured many diseases of plants by treating the seeds with various plant extracts (Ghewande 1989; Singh & Tripathi 2000) [12, 27]. Soil amendment with different plant parts for the control of soil-borne diseases has been demonstrated by various investigators (Singh & Rai 2000) [23].

Therefore, the present investigation indicates that the extract of *Curcuma malabarica* and *Hedychium spicatum,* on account of its fungitoxic, non-phytotoxic and remain fungitoxic, when placed in soil, under laboratory condition, may be recommended for large-scale field trials in order to explore the possibility of its use as herbal fungicide.

Conclusion

The main purpose of using plant extracts was to study their antifungal activity against the plant pathogens. In the present study, we use *Curcuma malabarica* and *Hedychium spicatum* extracts as natural fungicides and as eco-friendly means to control fungal plant diseases. As most of the plant extracts are readily available, environmentally safe, less risky for developing resistance in pathogen, and pathogen resurgence, has less or no phytotoxic effect on plant growth, seed viability and quality and above all, less expensive. All these observations and findings bring further evidence that the *Curcuma malabarica* and *Hedychium spicatum* plants extracts have the potential of becoming powerful and safe alternative means of disease control instead of the harmful chemical fungicides.

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