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Phytochemical and Antifungal Evaluation of Plant Extracts against *Alternaria brassicicola* (Schw.) Wiltshire Causing Black Leaf Spot of Cauliflower

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A B S T R A C T

The present study was conducted to detect the presence of phytochemicals in plant extracts pertaining to Meghalaya and their efficacy against *Alternaria brassicicola*, a causal agent of black leaf spot of cauliflower. Phytochemical screening of plant extracts studied showed that winged prickly ash and ginger had almost all the phytochemicals tested but least phytochemicals was observed in black pepper. Phytochemicals such as alkaloids and flavonoids were presence in almost all plant extracts. Aqueous extract of pitcher plant gave 100% inhibition of growth of pathogen at 10 and 15 % concentrations followed by garlic clove extract (83.78%) and pericarp extract of winged prickly ash (82.59%) at 15% concentration. Winged prickly ash, garlic, ginger, black pepper, sweet flag and pitcher plant showed highly effective against pathogen with increased in concentrations. Though derek, stinging nettle in all concentrations and wild brinjal at 5% were recorded less than 40% inhibition but showed a positive correlation between concentration and percent inhibition on growth of pathogen.

Introduction

Cauliflower (*Brassica oleracea* L. var. *botrytis*) is an important member of Cole crops which belongs to genus *Brassica* and family Brassicaceae. It is rich in minerals, nutrients and vitamins which makes it good for health. Besides thiamin, riboflavin, niacin, potassium, magnesium, iron and protein it has fairly high in vitamins A and C and folic acid content (Yamaguchi, 1983).

India is the second largest country next to China in cauliflower production in area (452000 ha) and production (8499000 MT) (Anonymous, 2017). A cruciferous crop including cauliflower is prone to attack by several insects and diseases that can significantly reduce yield and quality of crop. Many fungal, bacterial and viral plant pathogens attack the crop and amongst them fungal disease black leaf spot caused by two species of *Alternaria* are the most destructive one causing qualitative as well as quantitative

losses and are becoming a serious problem. However the disease caused by *A. brassicicola* is becoming more serious in the state.

Majority of the pesticides act like blunt weapons that kill organisms, in addition to target pests. Many of these substances contaminate the environment to a great extent. They can also enter the body of organisms, bio accumulate in the food chains and consequently affect the human health. There exists a direct relationship between the extent of pesticides used and signs and symptoms of illness due to exposure among farmers (Kishi *et al.,* 1995). The resistance of microorganisms against antimicrobial drugs is a major problem of recent times, which is increasing day by day (Cohen, 2000; Kumar *et al.,* 2013).

Plant extracts represent a rich source of antimicrobial agents and have been used by Indian system of medicine in preventive, promotive and curative applications. Extracts of various plant parts are found to be very effective against pathogenic seed borne fungi. Seed borne fungi can be efficiently achieved by using synthetic chemical fungicides but the same cannot be applied to grains for reasons of pesticides toxicity (Harris *et al.,* 2001). Plant-derived compounds such as hydroquinones and naphthoquinones (lapachol, juglone), sesquiterpenes (cinnamodial, capsidiol) and alkaloids (berberine) have shown diverse activities as antimicrobial and antifungal. An advantage to the approach of using ethnobotanical leads to identify compounds with antimicrobial activity (Galvan *et al.,* 2008). Medicinal plants are considered as the greatest pharmaceutical stores existing on the earth as they can produce eternal secondary phytochemicals having bioactive properties. These phytochemicals work efficiently to cure various diseases and illnesses since ancient times (Abdallah, 2011)

Therefore, in the present investigation major emphasis was to detect the presence of phytochemicals and to evaluate the efficacy of plant extracts which is an alternative approach to fungicides.

Materials and Methods

Isolation, identification and maintenance of pathogen

Leaf spot of cauliflower showing typical symptoms were collected from farmers' field of Ri-Bhoi district in Meghalaya. The leaves were then brought to the laboratory and microscopically examined to confirm the presence of the pathogen. After confirming for the presence of fungal spores, isolation was done by tissue segment method (Rangaswami, 1958) on Potato Dextrose Agar (PDA) medium. Cauliflower leaves showing characteristic of black leaf spot symptom were cut into small pieces of 2mm along with some healthy portions. The pieces were then surface sterilized with 1% NaOCl solution for one minute followed by serial washing in sterile distilled water thrice and blot dried with sterilized filter paper. The sterilized leaf bits were then aseptically transferred into 9cm Petri dishes containing sterilized PDA medium and incubated at 27±1°C. The fungus was purified by hyphal tip cut method. Culture was compared with the original description of the fungal pathogen and it was identified as *A. brassicicola*. The purified culture was maintained on PDA slants at 4˚C in refrigerator.

Collection of plant materials

The fresh plant materials of winged prickly ash (*Zanthoxylum khasianum*), wild brinjal (*Solanum xanthocarpum*), garlic (*Allium sativum),* ginger (*Zingiber officinale*), black pepper (*Piper nigrum*), sweet flag (*Acorus calamus*), derek (*Melia azadirach*) and pitcher

plant (*Nepenthes khasiana*) were collected from different places of Meghalaya, India and were used for the purpose of phytochemical analysis and its efficacy against *A. brassicicola*.

Preparation of plant extracts

The collected plants were surface sterilized with 1% sodium hypochlorite and then washed 3-4 times with distilled water followed by shade drying and the plant parts were then crushed into powder form and stored in polythene bags for further used.

Phytochemical analysis

Plant extracts was carried out for detection of phytochemical such as saponins, tannins, alkaloids, glycosides, flavonoids and phenol. The following test conducted was as follows:

Test for saponins: In this test 0.5 gm of extract was added in 10 ml of water, shaken for few minutes. Formations of frothing which persisted for 60-120 seconds, showed presence of saponins (Mojab *et al.*,2003).

Test for tannins: About 0.5 g of the dried powdered samples were boiled in 20 ml distilled water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added to the filtrate and was observed for blue-black or a brownish green coloration (Rashid *et al.,* 2013).

Test for alkaloids: A 0.2 g of the selected plant samples were added in each test tube and 3 ml of hexane were mixed in it, shaken well and filtered. A 5 ml of 2% HCl was then poured in a test tube having the mixture of plant extract and hexane. The test tube having the mixture was heated, filtered and few drops of picric acid in a mixture were added. Formation of yellow color precipitate indicates the presence of alkaloids. (Wadood *et al.,* 2013).

Test for glycosides: A 5 ml of dilute HCl was added in 0.5 gm of extract and boiled on water bath for 10 minutes. Solution was filtered and filtrate was extracted with benzene and mixed with ammonia solution. Red color was obtained in ammonia layer that indicated the presence of glycosides (Evans and Trease, 2008).

Test for phenol: About 0.5 g of dried powdered samples of each plant extract was dissolved in 2ml of distilled water and 1ml of iodine solution was added and observed for coloration. Formation of transient red color indicates the presence of phenol (Ansari, 2006).

Test for flavonoids: A 0.5 g of each selected plant extract was added in a test tube and 10 ml of distill water. 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of 1 ml concentrated H_2S0_4. Indication of yellow color shows the presence of flavonoids in each extract (Wadood *et al.,* 2013).

Efficacy of plant extracts against *Alternaria brassicicola*

Plant extracts were evaluated for their antifungal effects on growth *of A. brassicicola* by poisoned food technique (Nene and Thapliyal, 1979). Aqueous plant extract was prepared by taking 100gms of the desired plant material and washed them twice in running tap water and allowed them to dry for sometimes. Then they were subjected to washing again thrice with sterile distilled water and crush in a surface sterilized pestle and mortar by adding equal amount of sterile distilled water. After proper grinding, the extracts were squeezed through three layers of muslin cloth to extract the juice. The juices were then filtered it through a Whatman No. 42 filter paper. The filtrate was centrifuge at 15000rpm for 10mins. The supernatant was

then sterilized finally through bacteria proof membrane syringe filter (0.22µ) under laminar air flow. The final clear extracts prepared were the standard plant extracts of 100% concentration. These were evaluated at three different concentrations *viz.*, 5, 10 and 15% by adding required amount of extract to PDA medium. The plates containing PDA without any plant extract were maintained as untreated control. After solidification of media, all the plates were aseptically inoculated by placing in the centre a five mm mycelial disc obtained from a four days old actively growing pure culture and then incubated at 27±1°C. Each treatment was replicated thrice.

The tested fungus was recorded treatment-wise till mycelial growth gave fully covered in the untreated control plate. Percentage inhibition (I) of the pathogen was calculated by following the formula described by Vincent (1927).

$$I = \frac{C-T}{T} \times 100$$

Where,

I = Per cent inhibition of mycelial growth,

C = Growth in control and

T = Growth in treatment

Results and Discussion

The result (Table 1) showed the presence of alkaloids and flavonoids in almost all plant extracts. The least detected phytochemical was tannins. The presence of phytochemicals varied from one plant extract to another (Plate 1).

The study revealed that all plant extracts showed significant difference in per cent inhibition of radial growth of *A. brassicicola.* Pitcher plant extract gave a complete inhibition (100%) of mycelial growth at both 10% and 15% followed by garlic (83.78%), winged prickly ash (82.59%) and sweet flag (78.15%) (Table 2). The least per cent inhibition was recorded in stinging nettle (15.19%) at 5% concentration (Figure 1 and Plate 2). Most of the plant extracts showed more than 40 per cent inhibition. There was positive correlation between concentration and growth inhibition percentage in all the plant extracts.

Table.1 Phytochemical analysis of the extracts

Sl. No.	Plant	saponins	tannins	alkaloids	glycosides	flavonoids	phenol
1	Winged prickly ash	+	-	+	+	+	+
2	Wild brinjal	-	-	+	+	+	+
3	Garlic	-	-	+	+	+	+
4	Ginger	+	-	+	+	+	+
5	Black pepper	-	-	-	+	+	+
6	Sweet flag	+	-	+	+	+	-
7	Derek	+	+	+	-	+	-
8	Pitcher plant	+	+	+	-	+	-
9	Stinging nettle	+	+	-	-	-	+

(+) indicates presence of phytochemicals
(-) indicates absence of phytochemicals

Table.2 *In vitro* efficacy of plant extracts against the growth of *A. brassicicola*

Sl. No.	Treatments	Growth (cm)*			Per cent inhibition over control		
		5%	10%	15%	5%	10%	15%
1	Winged prickly ash	3.53±0.09^{f} (2.01)	3.03±0.12^{fg} (1.88)	1.57±0.12^{f} (1.44)	60.74±0.98^{b} (51.20)	66.30±1.34^{c} (54.52)	82.59±1.34^{b} (65.37)
2	Wild brinjal	5.47±0.03^{d} (2.44)	5.30±0.12^{d} (2.41)	3.30±0.15^{c} (1.95)	39.26±0.37^{d} (38.80)	41.11±1.28^{e} (39.88)	63.33±1.70^{e} (52.74)
3	Garlic	1.70±0.12^{g} (1.48)	1.62±0.10^{h} (1.45)	1.46±0.11^{f} (1.40)	81.11±1.28^{a} (64.27)	82.04±1.13^{b} (64.94)	83.78±1.19^{b} (66.28)
4	Ginger	3.57±0.12^{f} (2.02)	2.88±0.11^{g} (1.84)	2.47±0.12^{d} (1.72)	60.37±1.34^{b} (50.99)	67.96±1.21^{c} (55.54)	72.59±1.34^{d} (58.44)
5	Black pepper	4.77±0.12^{e} (2.29)	3.97±0.15^{e} (2.11)	3.43±0.15^{c} (1.98)	47.04±1.34^{c} (43.30)	55.93±1.61^{d} (48.41)	61.85±1.61^{e} (51.86)
6	Sweet flag	3.53±0.03^{f} (2.01)	3.17±0.09^{f} (1.91)	1.97±0.22^{e} (1.57)	60.74±0.37^{b} (51.20)	64.81±0.98^{c} (53.62)	78.15±2.43^{c} (62.19)
7	Derek	6.53±0.09^{c} (2.65)	6.20±0.12^{c} (2.59)	5.67±0.13^{b} (2.48)	27.41±0.98^{e} (31.57)	31.11±1.28^{f} (33.89)	37.04±1.48^{f} (37.48)
8	Pitcher plant	1.54±0.18^{g} (1.43)	0.00±0.00^{i} (0.71)	0.00±0.00^{g} (0.71)	82.85±1.99^{a} (65.60)	100.00±0.00^{a} (89.47)	100.00±0.00^{a} (89.48)
9	Stinging nettle	7.63±0.09^{b} (2.85)	6.67±0.12^{b} (2.68)	5.73±0.07^{b} (2.50)	15.19±0.98^{f} (22.92)	25.93±1.34^{g} (30.59)	36.30±0.74^{f} (37.05)
10	Control	9.00±0.00^{a} (3.08)	9.00±0.00^{a} (3.08)	9.00±0.00^{a} (3.08)	0.00±0.00^{g} (0.52)	0.00±0.00^{h} (0.52)	0.00±0.00^{g} (0.52)
SE(m)		0.03	0.03	0.04	0.76	0.71	0.91
CD (p=0.05)		0.08	0.08	0.11	2.24	2.11	2.68

***Mean of three replications**

Note: **Figures in parentheses are square root transformed values for growth and figures in parentheses are arc sine transformed values for per cent inhibition over control**

Figure.1 Bar diagram showing the per cent inhibition of plant extracts against growth of *A. brassicicola*

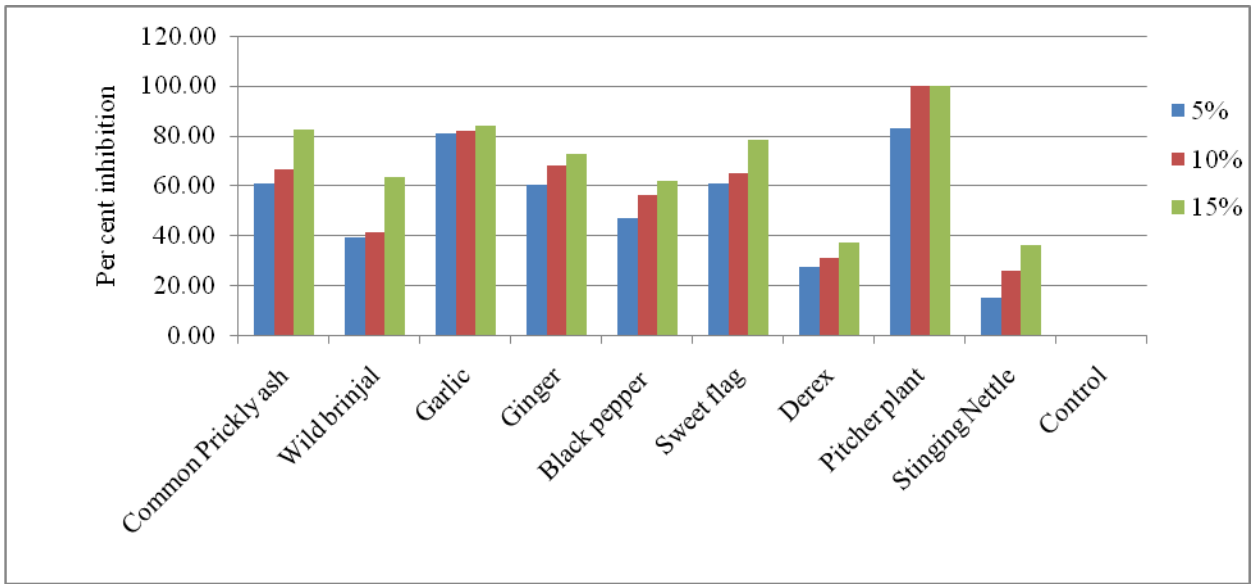
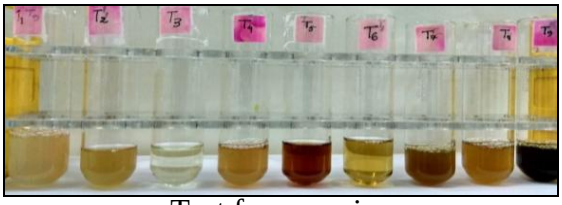
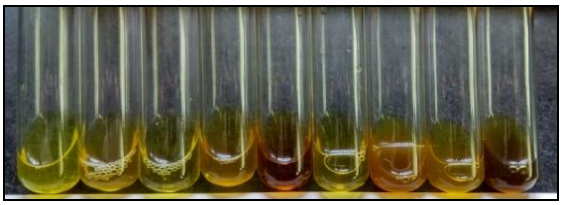


Plate.1 Phytochemical analysis of various plant extracts

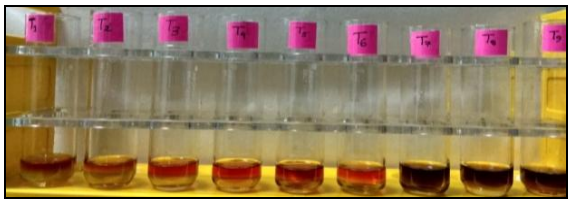


Test for saponins

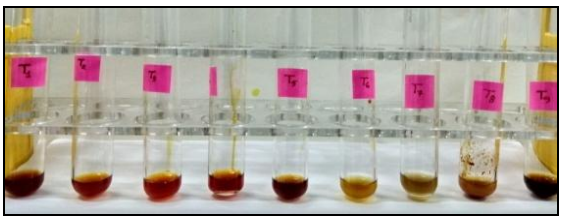
Test for tannins



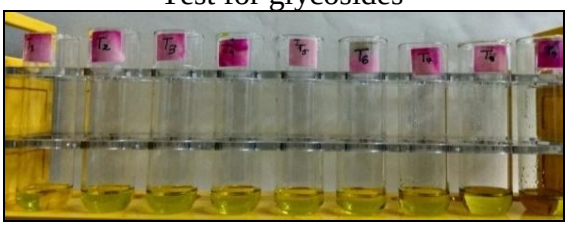
Test for alkaloids



Test for glycosides

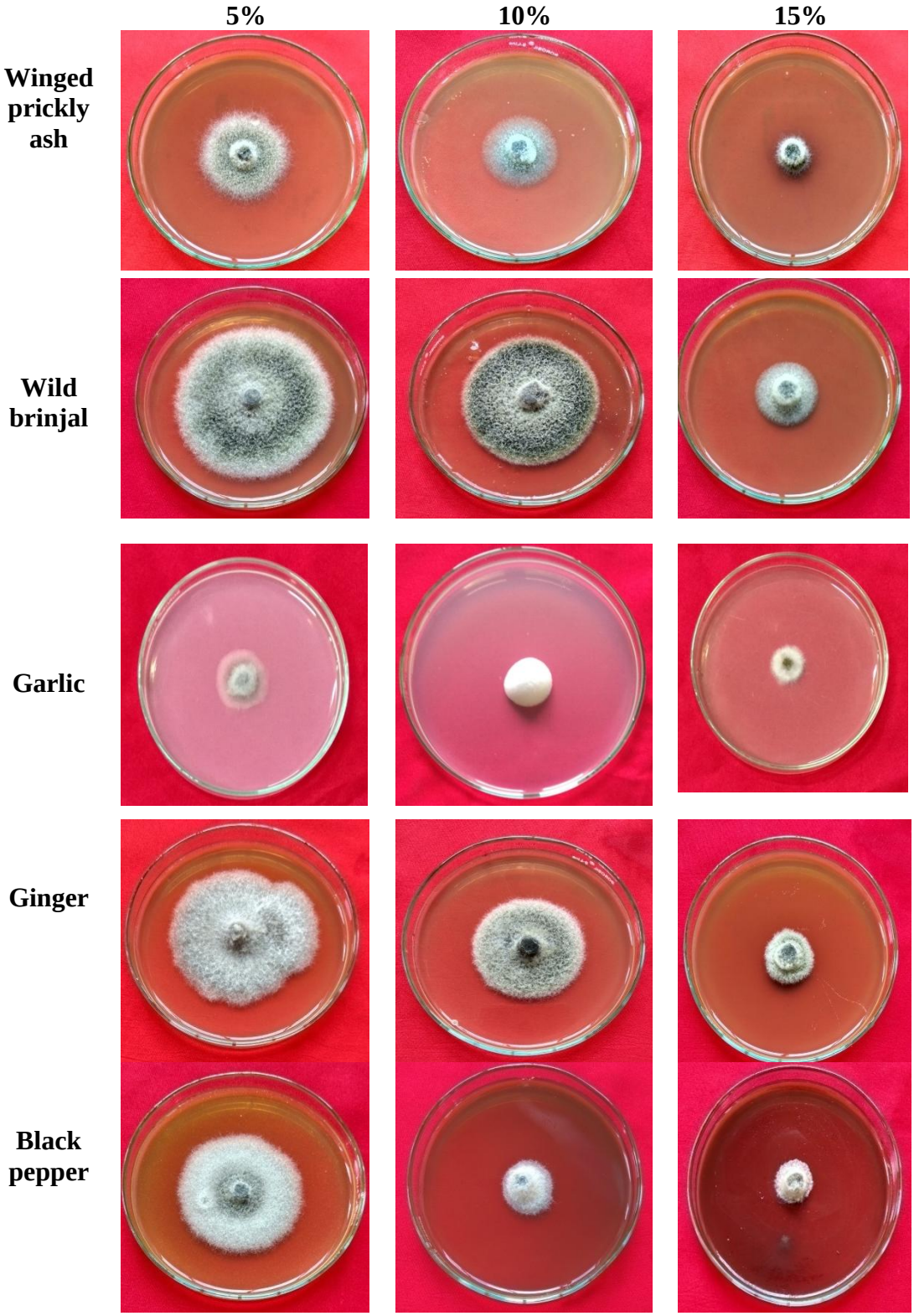


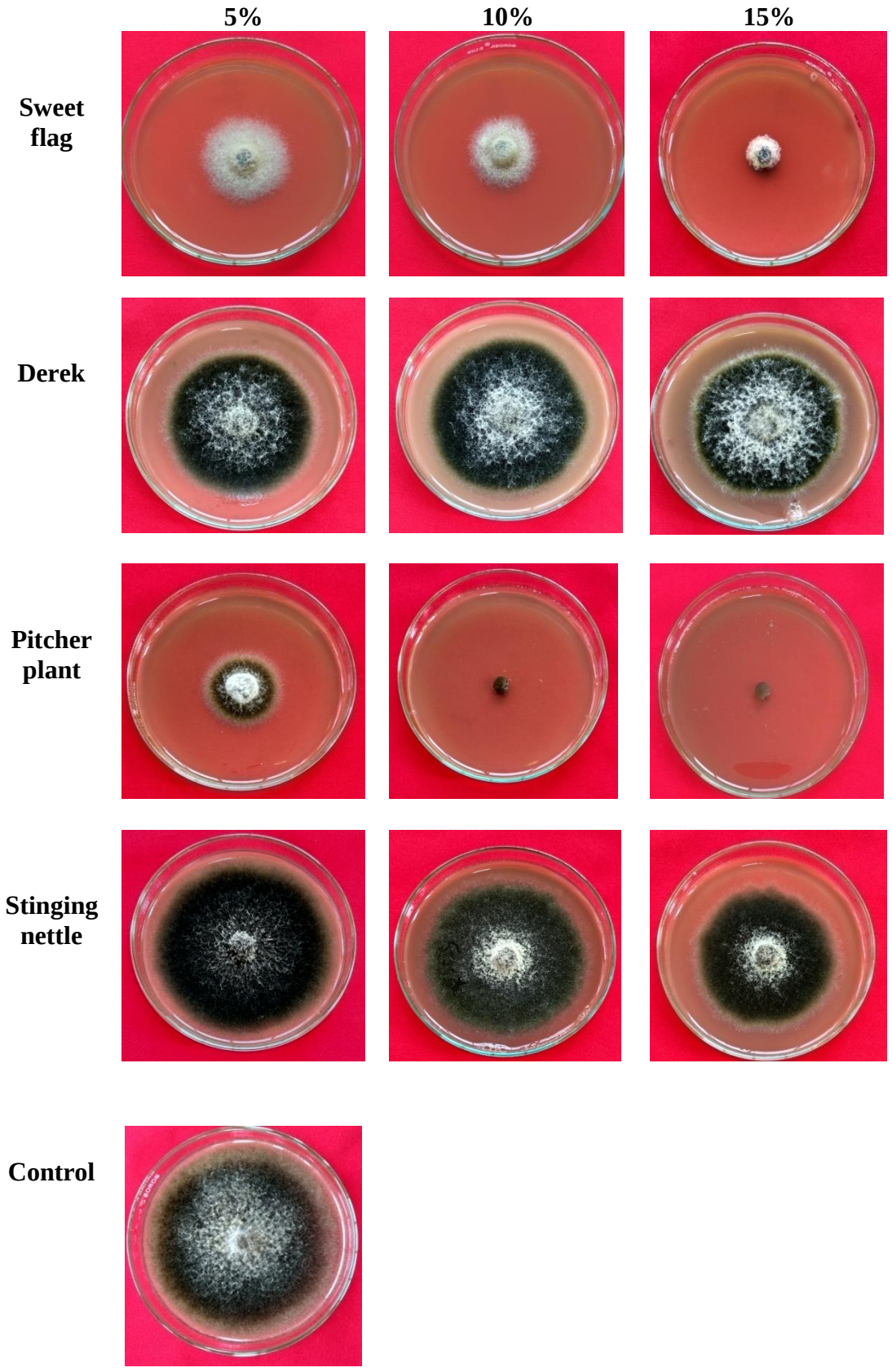
Test for phenol



Test for flavonoids

Plate.2 Efficacy of plant extracts against *A. brassicicola*




5%
10%
15%
Sweet flag
Derek
Pitcher plant
Stinging nettle
Control

Among all the phytochemical tested, alkaloids and flavonoids were found presence in almost all plant extracts *viz.,* winged prickly ash, wild brinjal, garlic, ginger, black pepper, and sweet flag. The least detected phytochemical was tannins which presence only in derek, pitcher plant and stinging nettle. The phytochemical screening of nine plants extract studied showed that winged prickly and ginger had almost all these phytochemicals tested *viz.,* saponins, alkaloids, glycosides, flavonoids and phenol but least phytochemicals was observed in black pepper and stinging nettle. Imam *et al.,* (2013) reported the presence of glycosides, flavonoids, saponins, tannins, polyphenolic compounds in sweet flag which is similar to the present findings.

Among the nine plant extracts evaluated at three different concentrations, pitcher plant extract gave 100% inhibition of growth at 10 and 15 % concentrations followed by garlic clove extract (83.78%) and pericarp extract of winged prickly ash (82.59%) each at 15% concentration. Winged prickly ash, garlic, ginger, black pepper, sweet flag and pitcher plant showed highly effective against *A. brassicicola* with increased in concentrations. However, derek, stinging nettle at all concentrations and wild brinjal at 5% were recorded less than 40% inhibition of growth of pathogen. The present finding is supported by findings of Sowjanya and Charay, (2012) who reported that *Allium sativum* was found to be most effective almost completely checking the mycelia growth at 10% concentration showing 83.09% inhibition against *Microsporum gypseum.* Similarly Shenoi *et al.,* (1998) evaluated that the plant extracts were less effective at lower concentrations but there was a positive correlation between concentration and growth inhibition percentage of *Alternaria alternata* causing brown spot of disease of tobacco. Mohammad *et al.,* also (2015) also reported that the potent inhibition of aqueous extract of *Melia azedarach*, *Cassia siamea* and *Morraya koenigii* on the growth of *Aspergillus niger* is due to presence of phenolics, alkaloids, flavonoids and tannins**.** It was also reported that these bioactive molecules give resistance to plants against pests and pathogenic infections by Dixon (2011).

In conclusion, phytochemical analysis found that almost all plant extracts revealed the presence of alkaloids, flavonoids, phenols, glycosides, saponins and tannins. Aqueous fresh extracts of pitcher plant at all concentrations showed maximum per cent inhibition of *A. brassicicola*. Out of Nine plant extracts evaluated, seven of them showed highly effective in inhibiting the growth of the pathogen. Plant extract could be recommended as one of the component into IDM programmes.

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