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Effect of selected botanicals against *Alternaria solani* and *Meloidogyne incognita* (J_2)

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Abstract
The antimicrobial activities of locally available plant extract (leaves, fruit, seeds, and barks) which are easily found around the surrounding of fields or some which are already being tested in the lab condition on some fungi are being used. Ten different plants were selected for testing; these plants are *Artocarpus heterophyllus, Aegle marmelos, Citrus bergamia, Cannabis sativa, Epipremnum aureum, Albizia lebbeck, Callistemon, Tecoma stans, Plumeria rubra* and *Bombax ceiba*. These plants have shown antifungal as well as antimicrobial property against *Alternaria solani* and *Meloidogyne incognita*. These plant extracts have shown inhibition activity against A. *solani.* Among all the plants *Plumeria rubra* and *Aegle marmelos* were observed to show the most effective results against *A. solani*. While *Cannabis sativa* and *Epipremnum aureum* were observed to show the most effective results against *M. incognita*. The above mentioned plants can be utilized against the management of disease caused by *A. solani* as well as against *M. incognita* (J_2).

Keywords: antimicrobial activity, *Alternaria solani*, *Meloidogyne incognita*, plant extract

Introduction
A number of plant species have been reported to possess natural substances that are toxic to plant pathogenic fungi (Goussous *et al*., 2010) [12]. Numerous researches have been done to study the effect of plant extracts on bacteria and fungus as they were found to contain phytochemical such as alkaloids, tannins, saponins, flavonoids, diterpenes, glucosinolates, acetylenes and thienyls (Goswani *et al*., 1986, Chitwood, 2002) [11, 8]. Medicinal plant material represents a rich source of antibacterial and antimicrobial agents (Mahesh and Satish, 2008; Adnan *et al*., 2010) [17, 4]. Natural plant products are important sources of new agrochemicals for the control of plants diseases (Kagale *et al*., 2004) [14]. Furthermore, biocides of plant origin are non-phytotoxic, systematic and easily biodegradable (Qasem and Abu-Blan, 1996) [22]. Plant extracts are known to reduce the population of pathogens and control the disease development, and these plant extracts have a potential as environment safe alternative and as components in integrated pest management programs (Bowers and Locke, 2004) [7].
Ten plants extracts viz: *Artocarpus heterophyllus, Aegle marmelos, Citrus bergamia, Cannabis sativa, Epipremnum aureum, Albizia lebbeck, Callistemon, Tecoma stans, Plumeria rubra* and *Bombax ceiba* were used for the testing of their fungal effect against fungal pathogen and plant parasitic nematode.
Alternaria solani is a fungal pathogen that produces a disease in tomato and potato plants called early blight (Ellis and Martin) Sorauer (Abada *et al*., 2008) [1]. The pathogen produces distinctive "bullseye" patterned leaf spot and can also cause stem lesions and fruit rot on tomato and tuber blight on potato. Despite the name "early" foliar symptoms usually occur on older leaves as well. If uncontrolled, early blight can cause dry rot of tubers and result in the reduction of both quantity and quality of marketing tubers. (Nnodu *et al*., 1982) [19]
Meloidogyne incognita is a soil-dwelling microscopic roundworm. They are commonly known as "root-knot nematode". It attacks the root of plants and sets up feeding location, where it destroys the normal root cells and establishes giant cells. The roots then become gnarled forming galls, hence the term "root-knot" nematode. The above ground effects of root parasitization, though non-specific, can be recognized as lack of vigour, stunted growth, yellowing of leaves. They are adaptive to parasitize on large number of plants and over 2000 wild and cultivated plant species are reported to be affected (Hussey and Janssen, 2002) [13].

Materials and Methods
Isolation of *Alternaria solani*
Potato plants showing typical symptoms of fungal attack were collected from the field (Plate 1). The causal fungus was then isolated by adopting the standard tissue isolation technique.

Later, the bit of fungal growth was transferred to PDA slants for purification, identification and maintenance of the culture. Plates were incubated for three days in room temperature.

Identification of fungus

Isolated fungus were identified by using of 10x and 40x magnifications on the microscope, hyphae, conidia, conidiophores and some other morphological characters including growth pattern, colony texture and growth rate of the colonies on PDA (Promputtha *et al*., 2005) [21]. Standard literature was referred for identification of pathogen (Ellis, 1976; Barnett and Hunter, 1972; Singh *et al*., 1991; Sutton, 1980) [9, 24]. The fungus was identified as *A. solani*, based on the morphological characteristics.

Collection of plant material

Six important medicinal plants viz: *Artocarpus heterophyllus, Aegle marmelos, Citrus bergamia, Tecoma stans, Plumeria rubra* and *Bombax ceiba* were collected form SHUATS campus and were found to possess antimicrobial and antifungal activity in accordance with the available literature. The plants were then washed with tap water as well as surface sterilized with 1% sodium hypochlorite and thereafter washed with distilled water. Leaf material were weighted at 10 gram and cut into small pieces, 10 ml water was added, and the leaves were grinded using mortar and pestle, the solution of the leaf were then filtered out using muslin cloth, the extract was centrifuged at 4000rpm from about 10 minutes.

Preparation of plant extract and screening

Plant extract was prepared with the help of a cork borer and 10 ml of plant extract were added with 90 ml of distilled water and sterilized at 15 lb. pressure. Antifungal effect of plant extract against *Alternaria solani* was determined by using Poison Food Technique in lab condition. The sterilized botanical extracts were poured into sterilized Petri-plates in laminar airflow to Petri-plats. After cooling down the plates, freshly grown *Alternaria solani* was introduced in the middle of the plates using a 5mm cork borer. Control plates were maintained and discs were inoculated to germinate in the BOD for 24, 48 and 72 hours and incubated at 28±2 °C in the BOD. Radial growth of mycelium were measured at every 24 hours interval for 4 days and compared with the result of the control. The following formula of percent inhibition was applied for each fungus in treatment (Vincent, 1947) [27].

$$\text{Per cent inhibition} = \frac{Y-Z}{Y} \times 100$$

Where Y= Mycelial growth in control
Z= Mycelial growth in treatment

Collection of *Meloidogyne* spp egg and screening:

The experiment was carried out by clearing the top soil with a spade after which the nematodes in the root-system were uprooted gently, roots were founds with moderate infection i.e. 11-30 galls (Plant 4). The infected roots were dipped in water and washed gently to remove soil. The galls were collected, washed properly for the collection of egg masses of *M. incognita* and placed in a glass cavity slide, transfer it further with 50% concentration of different extracts of botanicals and prepared by diluting the standard solution (S) with distilled water. They were then kept at room temperature allowing the eggs to hatch for 24, 48, 72 hours, while the juvenile released in distilled water were served as control (Alam, 1985) [2]. The suspension from each glass cavity was first transferred to nematode counting dish, where second stage juveniles of *M. incognita* were counted after every 24 hours. The number of hatched juveniles was counted using a stereoscopic microscope.

Table 1: List of the botanicals and its uses

Name of the plants	Family	Common name	Parts used	Medicinal uses
Tecoma Stans	*Bignoniaceae*	Yellow Bells	Leaves	It is effective as a diuretic, tonic, anti-syphilitic and vermifuge. It is used in the treatment of diabetes and also stomach pain.
Aegle marmelos	*Rutaceae*	Bael	Leaves	The leaf extracts were found to have fungicidal activity against various dermatophytic fungi. They are also antibacterial, antiviral, gastroprotective and anti-diarrheal effects.
Artocarpus heterophyllus	*Moraceae*	Jack Fruit	Leaves	They possess antibacterial, anti-inflammatory, antidiabetic and are rich in artocarpesin, oxydihydroartocarpesin, artocarpin, cycloartenol, betulinic acid, heterophylla which are helpful for boils, skin diseases and snake bites, etc.
Citrus bergamia	*Rutaceae*	Bergamot	Leaves	The essential oil of bergamot is used in numerous treatments such as high fever, intestinal worm, skin infection, gastritis infection, flu and also possesses antifungal properties.
Plumeria rubra	*Apocynaceae*	Plumeria	Leaves	The whole plant is used for cholera and indigestion, while the roots are used as laxative, thermogenic, astringent and the leaves are used for inflammation and swelling.
Bombax ceiba	*Malvaceae*	Red cotton tree	Leaves	They are antibacterial and antifungal due to the presence of tannins. Used in treating piles, constipation, and urinary disorder.
Cannabis sativa	*Cannabaceae*	Hemp	Leaves	The oil from the plants is found to be containing antimicrobial as well as antibacterial properties. They are also effective in treating arthritis, migraine, glaucoma and anorexia.
Epipremnum aureum	*Araceae*	Devil's ivy	Leaves	It is used for pest contrail as it contains calcium oxalate. *E. auerum* also have antibacterial, anti-termites and antioxidant properties. They are used for treating burns, sores.
Albizia lebbeck	*Fabaceae*	Siris	Leaves	They are found to have antibacterial and antimicrobial properties. They are used to treat Asthma, kidney disease and acne.
Callistemon	*Myrtaceae*	Bottlebrushes	Leaves	The plant has been reported to contain antibacterial, antifungal, antioxidant activities. And they are also a rich in phenolics, triterpenoids, flavonoids, saponins, steroids, alkaloids, tannin and amino acids.

Result and Discussion

Fungus (*Alternaria solani*)

Data presented in Table 2 and depicted in Figure 1 reveals the result that the plant extracts were effective in significantly reducing the growth of mycelia as compared with the control plates. However, all plants showed antifungal activity against *Alternaria solani*. The fungal growth of T_1 (*Tecoma stans*) at 48 hours it was significantly reduced from T_0 (Control), T_2

(*Aegle marmelos*), T_3 (*Artocarpus heterophyllus*), T_4 (*Citrus bergamia*) and T_6 (*Bombax ceiba*). At 72 and 96 hours T_5 (*Plumeria rubra*) was significantly reduced from T_0, T_4 and T_6 where as it was found non-significant from T_3, T_1 and T_2. Therefore from the given result we observed that T_5 (*Plumeria rubra*) was more effective in reducing the fungal growth of *A. solani* as compared to the other plant extracts.

It we observed that some of the plant extracts have shown very strong inhibition against the mycelium growth. Similar effects of various other plant products effective against *Alternaria* spp. were reported by several workers (Latha *et al*., 2009; Goussous *et al*., 2010) [16, 12]. The inhibitory effect of the plant extracts may be due to direct toxic effect on the pathogen (Vijayan, 1989) [26]. Though the suppression of the disease by plant products may be due to the active principles present in plant extracts and it may either act directly (Amandioha, 2000) [3] or indirectly systemic resistance in host plants resulting in a reduction of the disease development (Kagale *et al*., 2004) [14].

Table 2: Radial growth (mm) of *Alternaria solani* as affected by treatments

Botanicals	Extract Concentration	Radial mycelium Growth of *Alternaria solani* after Incubation (mm)				Per cent inhibition
		24h	48h	72h	96h	
Control (T_0)	10%	0.6	1.4	2.3	3.6	0
Tecoma Stans (T_1)	10%	0.4	0.6	1.6	2.4	36.87%
Aegle marmelos (T_2)	10%	0.65	1.3	1.55	2.05	29.80%
Artocarpus heterophyllus (T_3)	10%	0.6	1.3	1.75	2.5	22.22%
Citrus bergamia (T_4)	10%	0.65	1.25	2.2	2.85	12.12%
Plumeria rubra (T_5)	10%	0.5	0.85	1.2	1.6	47.47%
Bombax ceiba (T_6)	10%	0.75	1.15	2	3.0	12.63%
C.D at 5%		N.S	0.322	0.673	0.837	

Plate 1: Leaf spots caused by *Alternaria solani on Solanum tuberosum*.

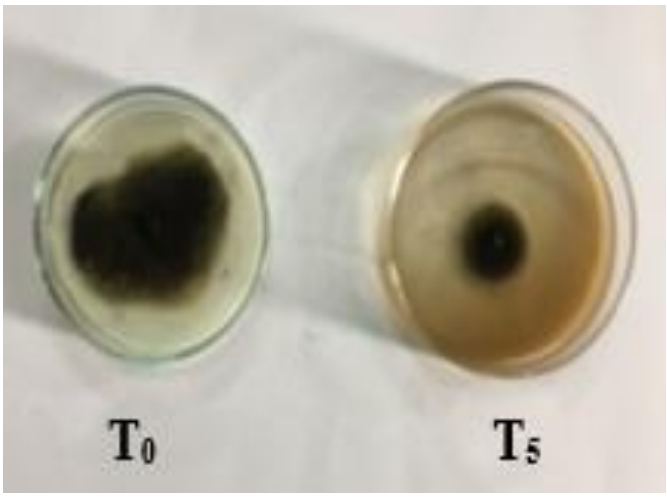

Plate 2: Mycelia growth of *Alternaria solani* on untreated (T_0) and *Plumeria rubra* (T_5) treated PDA

Plate 3: *Alternaria solani* under microscope.

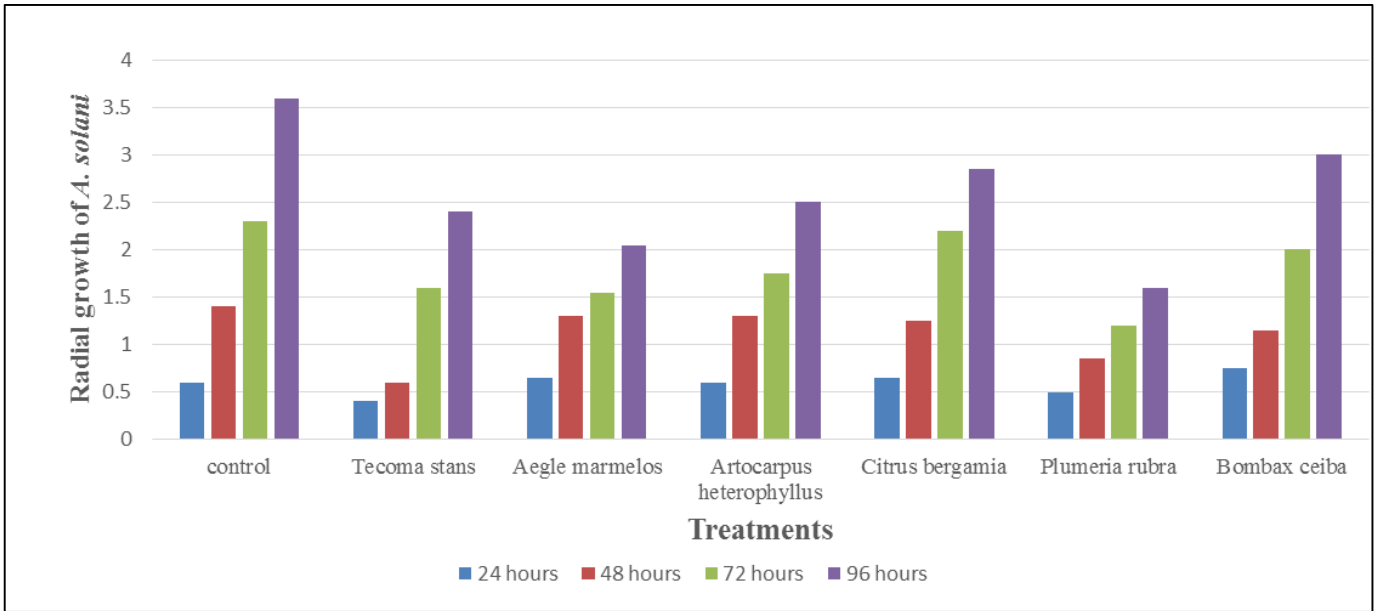

Fig 1: Effect of the botanicals on the radial growth (mm) of *Alternaria solani*

Nematode (*Meloidogyne incognita)*

Date presented in Table 3 and depicted in Figure 2 reveals that the result indicated that all tested plant extract caused a significant increase in the mortality rate of hatching of J_2 juveniles when exposed to the botanicals. Almost all the plant extract caused a decrease in the hatching of juveniles after 24 hours of exposure, but out of all T_4 (*Cannabis sativa*) observed to significantly reduced form T_0 (Control), T_8 (*Tecoma stans*), T_2 (*Aegle marmelos*), T_7 (*Callistemon*), T_1 (*Artocarpus heterophyllus*), T_9 (*Plumeria rubra*), T_3 (*Citrus bergamia*) and T_6 (*Albizia lebbeck*), while T_5 (*Epipremnum aureum*) and T_4 were non-significant form each other. Form both 48 and 72 hours it was observed that T_4 was significantly reduced form T_0, T_8, T_2, T_7, T_1, T_9, T_3, T_6 and T_5, while T_9, T_3 and T_6 were non-significant form each other. Through the observed result T_4 (*Cannabis sativa*) was found to be the most effective in reducing the hatching of M. incognita (J_2). With the entire test from the plant extracts it has proven to control the hatching of M. incognita effectively.

Table 3: Effect of the botanicals on the emergence of *Meloidogyne incognita* (J_2).

Plants	Extract Concentration	No. of eggs at 0 (zero) day	Release Juveniles of *Meloidagyne incognita* after exposing the eggs to the botanicals. (mean of the three replication)			Per cent inhibition
			24 hours	48 hours	72 hours	
Control (T_0)		794	779.67	765.34	611.34	
Artocarpus heterophyllus (T_1)	50%	977	152.67	78.34	73	85.90%
Aegle marmelos (T_2)	50%	855	277.34	188.67	177.33	70.16%
Citrus bergamia (T_3)	50%	884	6.33	2	1.33	99.55%
Cannabis Sativa (T_4)	50%	717	0	0	0	100%
Epipremnum aureum (T_5)	50%	801	2.67	2.67	2.33	99.64%
Albizia lebbeck (T_6)	50%	722	4	2	1.34	99.66%
Callistemon (T_7)	50%	780	202.67	116.34	86.66	81.19%
Tecoma stans (T_8)	50%	815	408.34	216.34	128.67	65.06%
Plumeria rubra (T_9)	50%	804	60.33	25.34	15	95.33%
C.D at 5%		N.S	2.830	2.426	2.031	

Plate 4: Galls of *Meloidogyne incognita* (J_2) on the roots

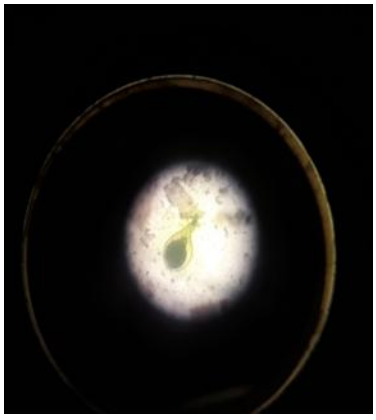

Plate 5: Egg masses of *Meloidogyne incognita* (J_2) under microscope.

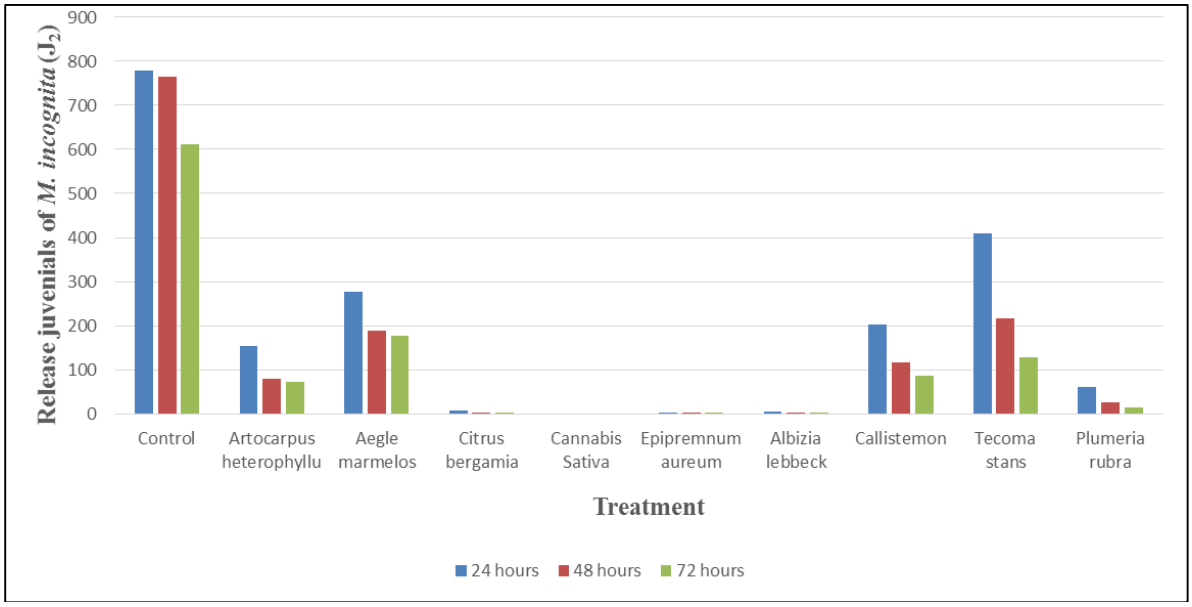

Fig 2: Effect of the botanicals on the emergence of *Meloidogyne incognita* (J_2).

Medicinal plants represent a rich source of antimicrobial as well as antifungal agents (Mahesh and Satish, 2008) [17]. Many of the plant materials are found in rural areas and are also used in traditional medicine (Mann *et al*., 2008) [18]. With its ever increasing need for more alternative eco-friendly approach to control the phyto pathogens, it was believed to be worthwhile to screen the antifungal activity effect of plants that are locally available (Bhardwaj, 2012) [5]. Plant extract of many higher plants has been reported to have properties such as antibacterial, antifungal and insecticidal properties under laboratory trials (Okigbo and Ogbonnaya, 2006; Shariff *et al*., 2006; Bouamama *et al*., 2006; Ergene *et al*., 2006; Kiran and Raveesha, 2006) [20, 23, 6, 10, 15].

The result through this study indicating that different activities of plant extracts on the mycelium growth of *A. solani* as well as on *Meloidogyne incognita* has shown significant and aggressively inhibition observed against both *A. solani* and *M. incognita*. But *Cannabis sativa* and *Plumeria rubra* were found to be effective and they represent a promising future in biological control against problem caused by *M. incognita* and *A. solani* respectively

Conclusion

Through this study it demonstrated that many plant extract e.g. *Artocarpus heterophyllus, Aegle marmelos, Citrus bergamia, Cannabis sativa, Epipremnum aureum, Albizia lebbeck, Callistemon, Tecoma stans, Plumeria rubra* and *Bombax ceiba* can be used as a bio control against different pathogens and nematodes. Thus, this method of control can contribute to minimizing the risk and hazards of toxic fungicides and chemicals, especially on vegetables produced for fresh consumption. It will be helpful in the formulation of ecofriendly control measures, which are cheap and can be formulated and applied by farmers (Yeni, 2011) [28].

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