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Research Article

ANTIMICROBIAL POTENTIAL OF *TRIDAX PROCUMBENS* PLANT EXTRACT AGAINST PHYTOPATHOGENIC FUNGI

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A B S T R A C T

The present study was carried out to evaluate antifungal activity of *Tridax procumbens* against phytopathogenic fungi. *Tridax procumbens* commonly called as '*coat button'*. In our study a total 25 samples were collected from infected plants like Rice, Citrus, Cucumber, Pulses and Ladies Finger. The phytopathogenic fungal strains resistant to various choosen chemical fungicides subjected to action of *Tridax procumbens*. In the current study Aqueous crude extract and methanolic extract of leaves, stem and whole plant material of *Tridax procumbens* evaluated for effectiveness based on zone of growth inhibition against phytopathogenic fungi. Aqueous extracts has remarked antifungal properties. Leaf extract showing broad spectrum of action against chemical fungicides resistant strains of *Fusarium spp, Rizoctonia spp, Mucor spp, Penicillium spp* and *Aspergillus spp.* This study shown aqueous as well as organic extracts of *Tridax procumbens* could be applied as alternative remedy to synthetic pesticides for using in agro- industries.

INTRODUCTION

Tridax procumbens belongs to family *Asteraceae,* commonly known as *"Ghamara"* and in English commonly known as "*Coat button"* because of appearance of flowers. *Tridax procumbens* is a week, straggling herb about 12-24 cm long with few leaves with length 6-8 cm long and very long slender solitary peduncles a foot long or more. Plant is semi prostate annual or short lives perennial. It is weed of pasture and wide range of annual and perennial crop type. The leaf is simple, opposite exstipulate, ovate acute, inflorescence capitulum. *Tridax procumbens* has two types of flower, ray-florets and disc-florets, basal placentation, fruit is cypsela type. Fruit is narrowly ovoid to cylindrical, tapering to bland base, blackish brown pyrose with pale ascending. [10]

The plant is native of tropical America and naturalized in tropical Africa, Asia, and Australia. It is a wild herb distributed throughout India. Coat buttons is also found along roadsides, waste grounds, dikes, railroads, riverbanks, meadows, and dunes. Its widespread distribution and importance as a weed are due to its spreading stems and abundant seed production [5].

It has known for its number of pharmacological activities like hepato-protective activity, anti-inflammatory, wound healing, antidiabetic activity, hypotensive effect, immune-modulating property, bronchial catarrh, dysentery, and diarrhea to prevent falling of hair promotes the growth of hair, and antimicrobial activity against both gram-positive and Gram-negative bacteria. The leaf juice possesses antiseptic, insecticidal and parasiticidal properties, as a remedy against conjunctivitis and is used also to check hemorrhage from cuts, bruises and wounds insect repellent. It is also used as bio adsorbent for chromium [12].

Tannins have been reported to prevent the development of microorganism by precipitating microbial protein. The growth of many fungi, yeast, bacteria, and viruses were inhibited by this compound [14].

Pathogenic fungi are the main infectious agents in plants, causing alterations during developmental stages including post-harvest. In fruit and vegetables, there is a wide variety of fungal genera causing quality problems related to aspect, nutritional value, organoleptic characteristics, and limited shelf life. [2]. In some cases fungi are indirectly responsible for allergic or toxic disorders among consumers because of the production of mycotoxins or allergens.[13] Generally, phytopathogenic fungi are controlled by synthetic fungicides; however, the use of these is increasingly restricted due to the harmful effects of pesticides on human health and the environment. [8].

Bioagents (living antagonistic organisms) however, can be safer, more biodegradable and less expensive to develop as compared to synthetic fungicides [3].

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MATERIAL AND METHOD

Collection of Tridax procumbens plant and authentication: Whole healthy plant of *Tridax procumbens* is collected. Voucher specimen were deposited in department of Botony D. B. Science College, Gondia, taxonomically identified and authenticated as *Tridax procumbens* [11].

Collection of diseased plant material: whole plant were collected from infected rice plant showing leaf brown spots, sheath blight and leaf blast. Leaves showing curling from ladies finger plant were collected. Fruit and leaves from infected citrus plant showing characteristics black brown spots were collected [18].

Isolation of fungal pathogen: The infected plant part cut into small piece (2-3mm). Surface sterilization were done using 1% mercury chloride solution And using distilled water, made their suspension is prepared. Transfer 0.2 ml of this suspension to surface of Potato Dextrose Agar media plate. The plates were incubated at 30^0C for 2 to 7 days. After completion of incubation, fungal colonies were picked up and subculture on Potato Dextrose Agar slope and maintain in laboratory.[18]

Identification of fungal pathogen: The fungal pathogen were identified on the basis of morphological characteristics and cultural characteristics on different medias.[20].

- **Morphological examination: -**Each fungal isolates were subjected to cotton blue staining and there morphology is examined under microscope. .**[**18]
- **Culture characteristics: -**A loop full of pure culture of isolates was inoculated on the surface of PDA Agar, King B agar by spot inoculation method and incubated at 28^0C for 2-7 days. After incubation plates were observed for colony characteristics.[18]

Preparation of aqueous plant extracts: 25 g of shade dried, powder of plant materials were macerated separately with 50 ml of sterile distilled water using pestle and mortar. The macerate was first filtered through muslin cloth and then filtrate was centrifuged at 8,000 rpm for 15 min at room temperature. Supernatant was filtered through filter paper and heat sterilized at 120ºC for 30 min. The extract was preserved aseptically in a brown bottle at 4^0C until further use.[17]

Preparation of organic extract

25 g of shade dried, powder of plant materials filled separately in the thimble and extracted successively with 150 ml of methanol using a Soxhlet extractor for 48 h. The extracts were concentrated using rotary flash evaporator. After complete solvent evaporation, this solvent extract was weighed and preserved at 4^0C in airtight bottles until further use. [17]

Phytochemical Screening*:* Chemical tests were carried out on the aqueous extract of the powdered specimens using standard procedures to identify the phyto constituents.

Studies of efficacy of chemical fungicide and Tridax procumbens extract against phytopathogenic fungi

- ***Preparation of fungal plate:*** A loopful culture of phytopathogenic fungi was inoculated on the surface of potato dextrose agar (PDA) by spot inoculation method and incubated at 30^0C for 2 to 3 days.
- ***Preparation of experimental plate:*** 100 ml PDA was prepared in 6 different conical flasks and sterilized. After sterilization allow to cool and 5 ml, 10ml and 15 ml chemical fungicide added in 3 different flask and 0.001 ml of *Tridax procumbens* in another 3 flask. Mix thoroughly and pour 20 ml of this medium from each flask to each set of 5 plates. Another 100 ml sterile PDA medium without fungicide also dispensed in 5 different sterile Petriplate. All plates were allowed for solidification of medium.

After solidification of media, using 6mm gel borer. Disc of fungal growth lawn were picked up and transfer to the center of PDA supplemented with chemical pesticide and *Tridax procumbens* extract and another disc of same fungal culture was placed on plane PDA agar plate. Different plates were prepared for different organism. Then all plates were incubated at 30^0C until mycelium completely covered the agar surface in control plate. Observations of mycelium growth of test pathogen were recorded and % inhibition of pathogen was calculated by following formula.[7]

$$\%\mathbf{I} = \frac{\mathbf{100X(C - T)}}{\mathbf{C}}$$

I= Inhibition of mycelium growth
C= Growth of pathogen in control plates
T= Growth of pathogen in test plates.

Pot experiment: Experiment was carried out in pot. In this experiment treated seed were sown to test the effect of *Tridax procumbens* extract in field soil.

20 seeds per tray were sown and all trays were arranged in 6 randomized blocks. Seed treated with sterile distilled water was used as control. The treatment was similar to field condition. The plants were sprinkled by water at alternate day as required.

On the basis of seedling emergence percent seed germination was recorded. In addition to this shoot length and root length and maturity was recorded. On that basis rate of disease emergence was determined. [7]

RESULT AND DISCUSSION

Infected plant material was collected for occurrence of fungal isolates from plants like Rice, Citrus, Cucumber and Ladies Finger. The infected material blight rice sheath which shown disease sheath wilting shunted growth. Leaf of ladies finger, citrus and cucumber which shown brown spot yellowing of leaf due to fungal infection, and pulses leaf which shown white and brown batches and leaf curling.

Various fungal pathogens were reported in diseased material. Occurrences of different phytopathogenic fungi in diseased plant material were

Table 1 Occurrences of different fungal pathogen in diseased plant material

Sr. no	Infected plant material	Symptoms of disease	Fungal isolates
1	Rice sheath	Sheath wilting shunted growth	F1, F2, F3, F4, F5
2	Citrus leaf	Brown spotted of leaf	F6, F7, F8, F9, F10
3	Cucumber (fruit)	Brown spot on fruit	F11,F12,F13,F14,F15
4	Ladies finger (fruit)	Brown spot on leaf	F16,F17,F18,F19,F20
5	Pulses	White and brown patches on leaf and leaf curling	F21,F22,F23,F24,F25

From five disease plants materials total 25 fungal pathogen were isolated.

Fungal pathogens were identified on the basis of morphological and cultural characteristics of fungal isolates were summarized in following table.

Table 2 Cultural characteristics and identification of fungal isolates

Sr no	Isolates	Cultural characteristics on PDA	Cultural characteristics on King B media	Identification
1	F1,F2,F3,F4,F5	White colored thread like colonies	White colored thread like colonies	*Mucor spp*
2	F6,F7,F8,F9,F10	Mycelium growth is cottony and cream colored	Mycelium growth is cottony and cream colored	*Fusarium spp*
3	F11,F12,F13,F14, F15	Pinkish color	Pinkish color	*Rizoctonia spp*
4	F16,F17,F18,F19, F20	Green colored	Green colored	*Penicillium spp*
5	F21,F22,F23,F24, F25	Black colored	Black colored	*Aspergillus spp*

Determination of infectious routes of plant pathogens and their mechanisms of infection are of great importance in any disease control program. [19] Our finding coincides with the work of Elisane *et al.*, (2008). Who also isolated four strains from the contaminated soil. [6] They were identified as *Aspergillus sp*. [6] Sharma (2011) [15] isolated some fungi at Darjeeling tea garden soil and Sharma *et al.* (2011) reported some fungi from Lachung soil The result was compared with the study of other workers for the fungal strains *Alternaria alternate*, *Fusarium oxysporum, Fusarium solani, Aspergillus flavus,* and *colletotricum* spp.[15].

In our study *Fusarium* spp*, Rizoctonia* spp.,*Mucor* spp., *Penicillium* spp., *Aspergillus* spp. are found

Phytochemical screening: The Phytochemical screening revealed the presence of alkaloids, tannins, saponins, steroid, tarpenoid and flavonoid. Most of the secondary metabolites were identified in the polar (methanol and water) extract. . The concentration of polar metabolite is higher than non-polar metabolites in leaves. Alkaloid is one of the characteristics secondary metabolites. Flavonoids are known to be synthesized by plant in response to microbial infection. Hence it should be surprising that they have been found to be effective as antimicrobial substance against wide array of infectious agent.[9] tannin is also known as antimicrobial agent. They are water soluble polyphenol and precipitated proteins present in many plant food. Several fungal pathogens are present in the nature which causes many harmful diseases to the essential and beneficial plants. These pathogens are not only related to tissue damage, nacrosis and rottening to the plant parts but reduce the yield performances also. As medicinal plants are evolved with the pathogens, these pathogens are destroyed by the antimicrobial principles extracted from this plant

Antifungal potential of chemical fungicide

Antifungal potential of chemical fungicide were tested against 25 phytopathogenic fungal strains. Antifungal potential of chemical fungicides against phytopathogenic fungi was determined using percentage inhibition method similar to the method used by Sharma (2007). [16]

After testing of chemical antifungal agent check antifungal activity of *Tridax procumbens* against isolated phytopathogenic fungi. Prepared methanolic and aqueous extract of *Tridax procumbens*. Activity of these extract in following table

Table 3 Antifungal potential of chemical fungicide

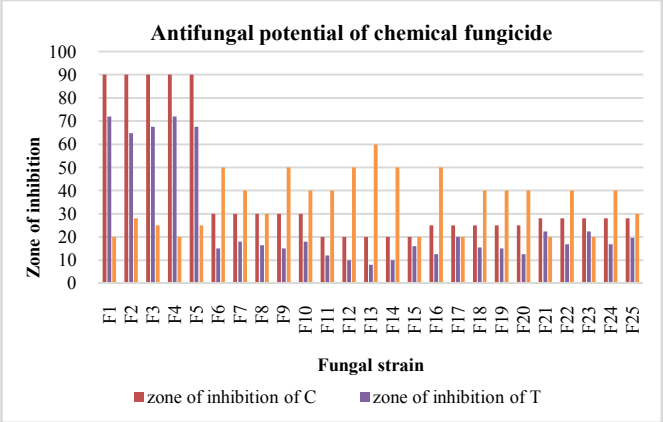

Table 4 Antifungal potential of *Tridax procumbens* extracts

Sr .no.	Fungal strain	% Growth Inhibition					
		Leaf extract		Stem extract		Whole plant extract	
		Aqueous	Methanolic	Aqueous	Methanolic	Aqueous	Methanolic
1	F1	27	25	17	15	22	20
2	F2	22	20	12	10	17	15
3	F3	29	27	17	15	20	18
4	F4	26	24	12	10	22	20
5	F5	22	20	10	8	18	16
6	F6	52	50	42	40	47	45
7	F7	47	45	42	40	44	42
8	F8	32	30	22	20	27	25
9	F9	62	60	52	50	57	55
10	F10	52	50	42	40	47	45
11	F11	52	50	47	45	44	42
12	F12	37	35	32	30	34	32
13	F13	42	40	37	35	39	37
14	F14	47	45	42	40	44	42
15	F15	32	30	27	25	29	27
16	F16	22	20	17	15	19	17
17	F17	32	30	20	28	30	28
18	F18	27	25	22	20	24	22
19	F19	32	30	27	25	29	27
20	F20	42	40	32	30	37	35
21	F21	62	60	52	50	57	55
22	F22	52	50	50	48	51	49
23	F23	47	45	42	40	43	41
24	F24	42	45	37	35	39	37
25	F25	42	40	37	35	39	37

In vivo effect of *Tridax procumbens* on fungal pathogen, studied using pot experiment. Efficiency of plant extract to control dropping of incidence of disease and effect on seedling growth of rice plant in presence of different phytopathogenic fungi. Efficacy of plant extract was summarized in following table.

Table 5 Efficacy of plant extract against *Fusarium* species in vivo condition

Sr no	Treatment	Percentage of seed germination	Shoot length in cm	Root length in cm
1	Positive control	85	10	3
2	Negative control	30	6	1
3	Leaf extract	90	9	2
4	Stem extract	60	7	1.2
5	Whole plant extract	80	7.5	1.5

Table 6 Efficacy of plant extracts against *Rizoctonia* species in vivo condition

Sr no	Treatment	Percentage of seed germination	Shoot length in cm	Root length in cm
1	Positive control	85	10	3
2	Negative control	30	5	0.7

3	Leaf extract	80	8	1.5
4	Stem extract	50	6	0.5
5	Whole plant extract	60	7	1.0

Table 7 Efficacy of plant extract against *Mucor* species in vivo condition

Sr no	Treatment	Percentage of seed germination	Shoot length in cm	Root length in cm
1	Positive control	85	10	3
2	Negative control	30	7	1
3	Leaf extract	70	8.0	1.7
4	Stem extract	50	7.2	1.2
5	Whole plant extract	60	7.5	1.5

Table 8 Efficacy of plant extract against *Penicillium* species in vivo condition

Sr no	Treatment	Percentage of seed germination	Shoot length in cm	Root length in cm
1	Positive control	85	10	3
2	Negative control	50	5	1.5
3	Leaf extract	100	9	2
4	Stem extract	80	7	1.0
5	Whole plant extract	90	8	1.5

Table 9 Efficacy of plant extract against *Aspergillus* species in vivo condition

Sr no	Treatment	Percentage of seed germination	Shoot length in cm	Root length in cm
1	Positive control	85	10	3
2	Negative control	20	5	1
3	Leaf extract	50	7	1.5
4	Stem extract	30	5	1.0
5	Whole plant extract	40	6	1.2

In Achrya S. *et al.* research 3 phytopathogenic fungal strain were taken for checked antifungal activity of *Tridax procumbens [1]*.In our study we taken 5 phytopathogenic fungi and checked activity of *Tridax procumbens*

CONCLUSION

Synthetic fungicides have been used for the control of plant disease, reduction in loss of harvest and decay of fruit and vegetables. Many fungal pathogens have developed resistance to most widely used chemicals. Resistance to fungicide and cost of chemical fungicide are major problems in pure disease control of agricultural origin, so there is need to develop alternative agent for the control of pathogenic fungal disease in plant. Natural products from plant have great potential as novel fungicide sources for controlling pathogenic fungi. Plant derived natural substances are considered as non-phototoxic compound and effective against plant pathogenic fungi. Aqueous as well as organic extract of *Tridax procumbens* could be applied as alternative industrial product to synthetic pesticide for using in agro industries and also to screen and develop selective and natural fungicide in the bio control of many agriculture plant pathogen causing drastic losses to crop.

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