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Managing chickpea wilt *Fusarium oxysporum* through use of biorationals

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ABSTRACT

The three agro-climatic zones of Jammu were surveyed and potential pesticidal plants / plant materials were collected. Their methanolic extracts were prepared following the standard procedure of refluxing, distillation and fractionation. They were assessed for antifungal activity against chickpea wilt, *Fusarium oxysporum* f.sp. *ciceri* by poisoned food technique. Among the 64 plant parts assessed, *Arisaema flavum* root / tuber extract (4.0 mm) recorded lowest radial growth, followed by *Boerrhavia diffusa* root extracts (6.0 mm) and *Arisaema flavum* stem + leaves extract (8.0 mm). Accordingly, *Arisaema flavum* roots / tubers extract also exhibited 95.55% and its leaves recorded 91.00% inhibition. *Boerrhavia diffusa* stem and root extracts again exhibited 94.5 per cent inhibition, while *Achyranthes aspera* root exhibited 91.11% inhibition. These extracts / fractions have great potential to be developed as botanical pesticide that can greatly benefit human beings in multifarious ways.

Key words: Botanicals, Fungicidal activity, *Fusarium oxysporum* f.sp. *ciceri*, Methanolic extracts.

INTRODUCTION

Among 37 pathogens attacking chickpea, in India, chickpea wilt, caused by *Fusarium oxysporum* f. sp. *ciceri*, is one of the most devastating and serious diseases of chickpea damaging it at all the stages, resulting in complete drying and causing significant losses. This disease is becoming increasing important in Jammu region of Jammu and Kashmir State. *Fusarium oxysporum* f.sp. *ciceri*, a soil borne pathogen colonizes the xylem vessels and completely block them to cause wilting, is one of the serious diseases of chickpea, causing losses upto 10-100% depending on environmental conditions (Sumitha and Gaikwad, 1995). Farmers resort to the application of chemical fungicides *viz*., Carbendazim, to manage this disease.

With the increased safety and health concerns, researchers are looking for alternatives to chemical pesticides, which are as good as them. Though pyrethroids, neem products are well established as pesticides, other plant products needs to be explored. Plethora of literature is available on fungicidal activities of several plant materials extracted with different solvents. Thousands of phytochemicals which have inhibitory effects on all types of microorganisms in vitro should be subjected in vivo testing to evaluate the efficacy in controlling the incidence of disease in crops, plants, and humans. Efficient collaborations with pharmacologists and medical doctors, plant pathologists and microbiologists are crucial to see the complete development of an interesting lead compound into an exploitable product (Gurjar, *et. al*., 2012).

Plant pathogenic fungi have caused devastating losses worldwide. Chemical fungicides are usually the first choice of the farmers, probably because of its ease of applicability and easy availability. Looking to the adverse impact of these chemical fungicides on environment, ecology and human beings, researches on finding effective alternatives need to be strengthened.

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Jammu province is rich in floral diversity as it covers three agro-ecological zones (sub-tropical, intermediate and temperate). All the three zones exhibit plants unique to its climatic conditions. Keeping all these facts in mind, research was conducted with the objective to assess the various extracts and fractions of few promising plants against chickpea wilt, *Fusarium oxysporum* f sp. *ciceri.* The present study is an attempt to reduce, the pesticidal load and its ill-effects in the environment and ecological system.

MATERIALS AND METHODS

All the three agro-ecological zones (sub-tropical, intermediate and temperate) of the Jammu province were surveyed for potential pesticidal plants (Table 1). The plants/ plant parts were collected from Bari Brahmana, (latitude-32.64°N, longitude-74.91°E and elevation-328.85 meters) Samba (latitude-32.55°N, longitude-75.11°E and elevation - 348 meters), Kathua (latitude - 32.39°N, longitude-75.52°E and elevation-387 meters), Mansar (latitude-32.70°N,

Table 1: Districts surveyed for collection of potential pesticidal plants.

Agro-climatic zone	Districts surveyed
Sub tropical	Jammu
	Samba
	Kathua
Intermediate	Samba
	Kathua
	Udhampur
	Reasi
Temperate	Udhampur

Table 2: Plants / plant parts collected from sub tropical zone.

Scientific name	Family	Plant part used
Achyranthes aspera	Amaranthaceae	Stem
		Leaves
		Roots
Alstonia scholaris	Apocynaceae	Leaves
		Bark
Boerhavia diffusa	Nyctaginaceae	Leaves
		Stem
		Roots
Calotropis procera	Ascelpiadaceae	Leaves
		Flowers
		Pods
Adhatoda vasica	Acanthaceae	Leaves
		Flowers
Murraya Koengii (L.) sprenge	Rutaceae	Leaves
		Fruits
Cannabis sativa	Cannabaceae	Leaves
		Stem
Butea frondosa	Leguminoceae	Leaves
		Bark
		Flowers
Euphorbia hirta	Euphorbiaceae	Leaves
		Stem + roots
Euphorbia geniculata	Euphorbiaceae	Whole plant

Table 3: Plants / plant parts collected from intermediate zone.

Scientific name	Family	Plant part used
Nerium indicum	Apocynaceae	Leaves
		Flowers
		Pods
Woodfordia fruticosa	Lythraceae	Leaves
		Flowers
		Roots
Nicotiana rustica	Solanaceae	Stem + leaves
Solanum surratense	Solanaceae	Leaves
		Fruit
Diplocyclos palmatus	Cucurbitaceae	Leaves + stem
		Fruits
Vitex negundo	Verbenaceae	Leaves
		Stem
		Inflorescence
Ficus palmata Forsk	Moraceae	Leaves
		Fruits
		Stem

longitude – 75.15°E and elevation – 666 meters) and Patnitop (latitude – 33.07°N, longitude – 75.34°E and elevation – 2024 meters), keeping the following points in mind.

- The plant is abundant in the area.
- It has some medicinal properties based on available literature and information gained from local people.

A total of 64 plants / plant parts were collected from all the three zones; sub tropical-23 (Table 2), intermediate-17 (Table 3) and temperate-24 (Table 4).

The collected plant material was shade dried and kept in plastic boxes for further use.

Preparation of plant extracts

Weighed quantity of shade dried plant material was crushed and kept in a round bottom flask. The solvent methanol was added to it in a volume just enough to immerse the bits. Refluxing was done by fitting the flask with a water condenser and boiling the set using heating mantle for 6 h (Fig 1). The extract was then filtered out of the flask and was concentrated by distillation process. This refluxing and distillation procedure was repeated thrice for the complete extraction of plant material. The quantity of extract obtained was also recorded (Fig 2). The methanolic extracts of all the collected plants / plant parts were then prepared by following the standard procedure of refluxing and distillation (Kumar *et al*., 2001).

Test fungus

Fusarium oxysporum f.sp. *ciceri* was isolated from infected chickpea plant using standard pathological techniques. The

Table 4: Plants / plant parts collected from temperate zone

Scientific name	Family	Plant part used
Diplazium esculentum	Athyriaceae	Leaves
Echinops cornigerus	Asteraceae	Leaves + Stem
Arisaema flavum	Araceae	Leaves + Stem
(Forsskal) Schott.		Roots / Tubers
Impatiens glandulifera	Balsaminaceae	Leaves
		Stem
Coccinia grandis	Berberidaceae	Fruits
		Leaves
Rumex nepalensis	Polygonaceae	Leaves + Stem
		Roots
Oxalis corriculata L.	Oxalidaceae	Leaves + Stem
Pinus wallichiana	Pinaceae	Bark
		Leaves
Cedrus deodara (Roxb.	Pinaceae	Bark
Ex. D. Do) G. Don		Leaves
Abies pindrow	Pinaceae	Bark
		Leaves
Fragaria virginiana	Rosaceae	Leaves + Stem
Verbascum thapsus	Scrophulariaceae	Leaves + Stem
Viburnum grandiflorum	Caprifoliaceae	Leaves
Malva neglecta Wallr.	Malvaceae	Leaves
		Roots
Inula royleana	Asteraceae	Leaves
		Flowers

Fig 1: Soxhlet's extraction unit.

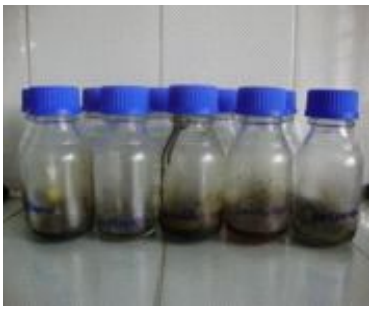

Fig 2: Extracts kept in reagent bottles.

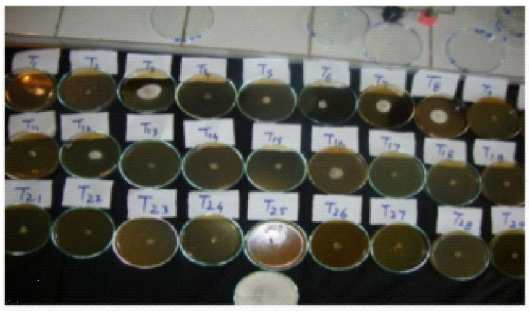

Fig 3: Antifungal assay by poison food technique.

media used was Potato Dextrose Agar (PDA). Pure culture of the test fungus was maintained. The assessment of fungitoxicity was done by poisoned food technique (Grower and Moore, 1962). Seven days old culture of the test fungus was used for the preparation of innoculum disc of 5 mm in diameter (Fig 3).

Antifungal assay

A volume of 0.5 ml of each concentration was asceptically poured into the petriplate followed by the addition of 9.5 ml of melted PDA and was swirled gently to achieve through mixing of the contents. Two controls, one treated with Carbendazim and the other completely untreated, were kept. In the control set, no extract was used. After the solidification of the media, one innoculum disc of the test fungus was aseptically inoculated upside down at the centre of the petriplate and incubated at 25 ± 2°C. The average diameter of the fungal colonies was measured on the 7th day of incubation and percentage of mycelial growth inhibition was calculated (Rao and Srivastava, 1994). All the extracts were initially assessed at 1000 ppm and almost all recorded complete inhibition. The concentration was then reduced to 500 ppm and again most of the extracts showed hundred per cent inhibition. Finally the extracts were assessed at 200 ppm concentration.

$$\text{Mycelial growth inhibition (\%)} = \frac{g_c - g_t}{g_c} \times 100$$

Where,

g_c = growth of mycelial colony in control set after incubation period substracting the diameter of innoculum disc.

g_t = growth of mycelial colony in treatment set after incubation period substracting the diameter of innoculum disc.

Based on the growth inhibition effects of these extracts on the test fungus, *F. oxysporum* f. sp. *ciceri,* few plants/ plant materials were selected for further evaluation.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) in a Completely Randomized Design after appropriate transformations as suggested by Gomez and Gomez (1984) before statistical analysis. The difference of two means between treatments exceeding Critical Difference (CD) value is significant (Panse and Sukhatme, 1978).

RESULTS AND DISCUSSION

Among the 23 plants/plant parts methanolic extracts, evaluated against *F. oxysporum*, *Boerrhavia diffusa* root extracts recorded lowest radial growth (6.0 mm), followed by *Boerrhavia diffusa* stem extract (8.0 mm) and *Achyranthes aspera* root extract (9.0 mm). Accordingly, *Boerrhavia diffusa* stem and root extracts again exhibited 94.5 per cent inhibition as evident from Table 5. This was followed by *Achyranthes aspera* root (91.11%) and stems (86.67%) extract. *Murrya koengii* fruits (75.56%) and leaves (70.00%) extract also exhibited more than 70% inhibition. All the treatments exhibited higher percent inhibition than that in control (water), while most treatments effect were comparable to that of control (Carbendazim). Few (*Boerrhavia diffusa* root, *Boerrhavia diffusa* stem and *Achyranthes aspera* root extract) performed even better than the carbendazim treatment, recording almost complete inhibition.

Woodfordia fruticosa root extract recorded lowest radial growth of 20 mm among the intermediate zone plants, followed by *Diplocyclos palmatus* fruit (21 mm) and *Nicotiana rustica* leaves + stem (22 mm) (Table 6). Likewise, *Woodfordia fruticosa* roots (77.78%), *Diplocyclos palmatus* fruits (76.67%), *Nicotiana rustica* leaves + stem (75.56%), recorded more than 75.00% inhibition. Though all the extracts recorded percent inhibition higher than the control (water), *Woodfordia fruticosa* root extract (20.0 mm radial growth), *Diplocyclos palmatus* fruits (21.0 mm), *Nicotiana rustica* leaves + stem (22.0 mm) and *Vitex negundo* leaves (30.0 mm), exhibited percent inhibition higher than carbendazim treatment (34.0 mm).

Lowest radial growth was recorded in *Arisaema flavum* root / tuber extract (4.0 mm) and stem + leaves extract (8.0 mm), among the temperate zone plants as evident from Table 7. This was followed by *Coccinia grandis* fruit (14 mm) and *Verbascum thapsus* leaves + stem extract (22 mm). Accordingly, *Arisaema flavum* roots/tubers extract also exhibited 95.55% and its leaves recorded 91.00% inhibition. This was followed by *Coccinia grandis* (84.44%) and *Verbascum thapsus* leaves + stem extract (75.56%).

Corroborating our study, few workers have reported the fungicidal activity of botanicals against chickpea wilt. Seed treatment with garlic leaf extract (Singh *et al*., 1979) and neem oil (Singh *et al*., 1980) are reported to reduce the

Table 5: Fungicidal activity of plants / plant parts of sub tropical zone

Scientific name	Plant part used	Radial growth (mm)	Per cent inhibition at 200 ppm
Achyranthes aspera	Stem	12.0	86.67
	Leaves	33.0	63.33
	Roots	9.0	90.50
Alstonia scholaris	Leaves	37.0	58.89
	Bark	34.0	62.22
Boerhavia diffusa	Leaves	31.0	65.56
	Stem	8.0	91.50
	Roots	6.0	94.50
Calotropis procera	Leaves	35.0	61.11
	Flowers	29.0	67.78
	Pods	45.0	50.00
Adhatoda vasica	Leaves	32.0	64.44
	Flowers	37.0	58.89
Murraya Koengii (L.) sprenge	Leaves	27.0	70.00
	Fruits	22.0	75.56
Cannabis sativa	Leaves	30.0	64.44
	Stem	35.0	61.11
Butea frondosa	Leaves	43.0	52.22
	Bark	41.0	54.44
	Flowers	39.0	56.67
Euphorbia hirta	Leaves	37.0	58.89
	Stem + roots	34.0	62.22
Euphorbia geniculata	Whole plant	33.0	63.33
Control (Carbendazim @ 20 ppm)		34.0	62.22
Control (Untreated)		90.0	-
S.E. (m)		0.27	1.98
C.D. at 5%		0.83	3.56

Table 6: Fungicidal activity of plants / plant parts of intermediate zone

Scientific name	Plant part used	Radial growth (mm)	Per cent inhibition at 200 ppm
Nerium indicum	Leaves	33.0	63.33
	Flowers	40.0	55.56
	Pods	30.0	66.67
Woodfordia fruticosa	Leaves	36.0	60.00
	Flowers	34.0	62.22
	Roots	20.0	77.78
Nicotiana rustica	Leaves + stem	22.0	75.56
Solanum surratense	Leaves	37.0	58.89
	Fruit	29.0	67.78
Diplocyclos palmatus	Leaves + stem	36.0	60.00
	Fruits	21.0	76.67
Vitex negundo	Leaves	30.0	66.67
	Stem	35.0	61.11
	Inflorescence	34.0	62.22
Ficus palmata Forsk	Leaves	55.0	38.89
	Fruits	50.0	45.55
	Stem	53.0	41.11
Control (Carbendazim)		34.0	62.22
Control (Untreated)		90.0	-
S.E. (m)		0.32	2.04
C.D. at 5%		0.78	4.25

Table 7: Fungicidal activity of plants / plant parts of temperate zone.

Scientific name	Plant part used	Radial growth (mm)	Per cent inhibition at 200 ppm
Diplazium esculentum	Leaves	44.0	51.11
Echinops cornigerus	Stem + Leaves	42.0	53.33
Arisaema flavum (Forsskal) Schott.	Stem + Leaves	8.0	91.00
	Roots / Tubers	4.0	95.55
Impatiens glandulifera	Leaves	34.0	62.22
	Stem	37.0	58.89
Podophyllum hexandrum	Fruits	14.0	84.44
	Leaves	29.0	67.78
Rumex nepalensis	Leaves + Stem	45.0	50.00
	Roots	35.0	61.11
Oxalis corriculata L.	Leaves + Stem	47.0	47.78
Pinus wallichiana	Bark	31.0	65.56
	Leaves	38.0	57.78
Cedrus deodara (Roxb. Ex. D. Do) G. Don	Bark	32.0	64.44
	Leaves	35.0	61.11
Abies pindrow	Bark	34.0	62.22
	Leaves	37.0	58.89
Fragaria virginiana	Leaves + Stem	30.0	66.00
Verbascum thapsus	Leaves + Stem	22.0	75.56
Viburnum grandiflorum	Leaves	47.0	48.89
Malva neglecta Wallr.	Leaves	37.0	58.89
	Roots	29.0	67.78
Inula royleana	Leaves	31.0	65.56
	Flowers	27.0	70.00
Control (Carbendazim)		34.0	62.22
Control (Untreated)		90.0	-
S.E. (m)		0.45	3.52
C.D. at 5%		0.92	6.86

pathogen. The antifungal effect of aqueous extracts of four plant species *viz*; *Azadirachta indica* A. Juss., *Datura metel* L. Torr., *Ocimum sanctum* L. and *Parthenium hysterophorus* L. was observed *in vitro* study. It was found that all four plant extracts at 40% concentration were effective in controlling the mycelial growth of *F. oxysporum* f. sp. *ciceri* (Irum, 2007). Leaf extract of *Azadirachta indica* at 100% conc. completely inhibited germination of pathogen spores (Singh and Hari Chand, 2004). *Azadirachta indica* leaf extract gave maximum inhibition (55.19%) of radial growth of *F. oxysporum* f. sp. *ciceri* (Hossain *et al*., 2013).

The antifungal or antibacterial activity of *Verbascum Thapsus*, *Woodfordia fruticosa*, *Arisaema flavum, Coccinia grandis*, have been proved by few researchers, which is in accordance with our study. *Verbascum thapsus* (Schrophulariaceae) better known as Mullein is a medicinal plant used in the treatment of inflammatory diseases, asthma, spasmodic cough, diarrhea and other pulmonary problems. *Verbascum thapsus* leaves were treated with n-hexane, chloroform, methanol, cold and warm water to obtained the extracts. In accordance with our studies, antifungal activity of *V. thapsus* was observed in its methanol extract (1000 µg mL^{-1}) against *Fusarium graminearum* and *Macrophomina phaseolina*. The antifungal studies of *Coccinia grandis* instant juice powder revealed a significant activity against fungal strains. It showed 2 to 5.1 mm zone of inhibition in the aqueous and solvent extract, due to the presence of phytochemicals (Elicy and Thilagavathi, 2017). Antibacterial activity of the flowers of *Woodfordia fruticosa* was assessed on different microorganisms (Kumar *et al*., 2015).

Arisaema flavum crude extract was active against all bacterial strains except *Staphylococcus aureus*. Maximum zone of inhibition (13.9 mm) was observed against *Pseudomonas picketti*. An average zone of 10-11 mm was observed against *Micrococcus leutus, Bacillus subtilis*, and *Salmonella Setubal*. Chloroform and methanol fractions of *Arisaema flavum* showed moderate activity against three strains while ethyl acetate fraction showed mild inhibition (9.6 mm) of *Micrococcus luteus* (Singh *et al*., 2004). Similarly, the crude extract of *A. flavum* was found active against different bacterial strains (three Gram positive and two Gram negative) (Bibi *et al.,* 2011).

CONCLUSION

These potentially active plant extracts (*Boerrhavia diffusa* root and stem, *Achyranthes aspera* root, *Woodfordia fruticosa* root, *Diplocyclos palmatus* fruit, *Nicotiana rustica* leaves + stem, *Arisaema flavum* root / tuber and stem +

leaves, *Coccinia grandis* fruit, *Vitex negundo* leaves and *Verbascum thapsus* leaves + stem) may be further evaluated in vivo and utilized for managing plant diseases in fields. They can also be further developed into botanical pesticides, in similar lines as neem and exploited commercially. This shall help greatly in mitigating the climate change impact up to some extent.

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