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Fungicidal activity of plants of Jammu region against chickpea wilt; *Fusarium oxysporum*

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Abstract
Jammu region is endowed with a rich floral diversity that can be explored for their potential pesticidal activity. In this pursuit, a project was formulated and plant materials were collected from all the agro-climatic zones of Jammu region (sub-tropical, semi-temperate and temperate). Collections were done based on their medicinal properties as informed by the local people. The plant materials were then shade dried and various fractions were prepared using different solvents (Hexane, Acetone and Ethanol) by refluxing, distillation and fractionation. Among the 36 different fractions of plants / plant parts assessed for toxicity against *Fusarium oxysporum*, *Arisaema flavum* roots / tubers hexane fraction recorded lowest radial growth of 5.0 mm followed by its acetone fraction (7.0 mm), *A. flavum* stem + Leaves hexane (7.0 mm) and *Boerrhavia diffusa* roots hexane fraction (7.0 mm). Accordingly, the per cent inhibition also varied, being the highest in *A. flavum* roots / tubers hexane (94.44%) followed by its acetone fraction (92.22%), *A. flavum* stem + leaves hexane fraction (92.22%) and *B. diffusa* roots hexane fraction (92.22%). These fractions have great potential to be further developed as botanical pesticide that may be utilized to reduce the pesticidal load in the environment. This shall also help in mitigating the climate change impact, to some extent.

Keywords: Fungicidal activity, *Fusarium oxysporum* f.sp. *ciceri*, Hexane, acetone and ethanol fractions

Introduction
The increased safety and health concerns have forced researchers to look for alternatives to chemical pesticides, which are as good as them. Pyrethroids, neem products are well established as pesticides, but other plant products need 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 [1].

Plant pathogenic fungi have caused devastating losses worldwide and chemical fungicides are usually the first choice of the farmers, probably because of its ease of applicability and easy availability. Chickpea (*Cicer arietinum* L.), commonly known as Bengal gram or gram, ranks 3rd amongst the global pulse cultivation. In India, it occupies an area of 4.9 mha, with production of 3.4 mT, and average productivity of 694 kg/ha. 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 important in the 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 [2]. Farmers resort to the application of chemical fungicides viz., Carbendazim, to manage this disease. Looking to the adverse impact of these chemical fungicides on environment, ecology and human beings, researches on finding effective alternatives needs to be strengthened.

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 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 fungicidal plants (Table-1). The plants / plant parts were collected from Bari Brahmana, (latitude – 32.64^0N, longitude – 74.91^0E and elevation – 328.85 meters) Samba (latitude– 32.55^0N, longitude – 75.11^0E and elevation – 348 meters), Kathua (latitude – 32.39^0N, longitude – 75.52^0E and elevation – 387 meters), Mansar (latitude – 32.70^0N, longitude – 75.15^0E and elevation – 666 meters) and Patnitop (latitude – 33.07^0N, longitude – 75.34^0E and elevation – 2024 meters), keeping the idea that the plant has some medicinal property as informed by the local people and literature available (Table 2).

Table 1: Districts surveyed for collection of potential pesticidal plants

S. No.	Agro-climatic zone	Districts surveyed
1.	Sub-tropical	Jammu
		Samba
		Kathua
2.	Intermediate	Samba
		Kathua
		Udhampur
		Reasi
3.	Temperate	Udhampur

Murraya koenigii (L.) Sprenge

Arisaema flavum Schott

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

Table 2: Plant / plant parts collected from all the three zones for fractionation and testing

S. No.	Scientific name	Family	Plant part used
1.	*Achyranthes aspera*	Amaranthaceae	Stem
2.			Roots
3.	*Boerhavia diffusa*	Nyctaginaceae	Stem
4.			Roots
5.	*Murraya Koengii* (L.) sprenge	Rutaceae	Fruits
6.	*Woodfordia fruticosa*	Lythraceae	Roots
7.	*Nicotiana rustica*	Solanaceae	Stem + leaves
8.	*Diplocyclos palmatus*	Cucurbitaceae	Fruits
9.	*Arisaema flavum* (Forsskal) Schott.	Araceae	Stem + Leaves
10.			Roots / Tubers
11.	*Coccinia grandis*	Berberidaceae	Fruits
12.	*Verbascum thapsus*	Schrophulariaceae	Leaves + Stem

Hexane, acetone and ethanol fractions of these 12 promising plants / plant parts were prepared.

Fungicidal activity of these fractions was assessed (Table-3) following the food poison technique.

Preparation of plant fractions

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. 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. The methanolic extracts of all the collected plants / plant parts were then prepared by following the standard procedure of refluxing and distillation [3].

Fractionation process

The methanolic extracts of these potential plants / plant parts were further subjected to fractionation using hexane first, followed by chloroform and acetone (based on their increasing polarity). The methanol extract was mixed with silica gel (Column chromatography) in a tray and left for 24 h, after which the mixture was transferred to a glass jar. Hexane was poured to fill three-fourth of the jar and distilled to obtain the hexane as residue left at the bottom of distillation flask. This process of adding hexane, leaving for 24 h followed by distillation was repeated thrice. After hexane, the same procedure was followed using acetone and then ethanol to obtain their respective fractions.

Soxhlet's extraction unit

Extracts kept in reagent bottles

Test fungus: *Fusarium oxysporum* f.sp. *ciceri* was isolated from infected chickpea plant using standard pathological techniques. The media used was Potato Dextrose Agar (PDA). Pure culture of the test fungus was maintained. The assessment of fungitoxicity was done by the poisoned food technique [4].

Innoculum disc: Seven days old culture of the test fungus was used for the preparation of innoculum disc of 5 mm in diameter.

Antifungal assay: A volume of 0.5 ml of fraction (200 ppm) 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, as shown in tables. In the control set, no fraction was used. After the solidification of the media, one innoculum disc of the test fungus was aseptically inoculated upside down

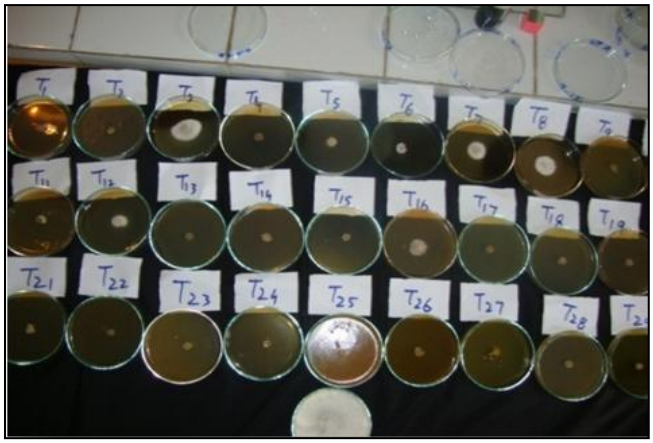
at the centre of the petriplate and incubated at 25 ± 2^0C.
The average diameter of the fungal colonies was measured on the 7th day of incubation and percentage of mycelial growth inhibition was calculated [5].

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

Where,

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

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



Antifungal assay by poison food technique

Statistical analysis
The data were subjected to analysis of variance (ANOVA) in a Completely Randomized Design after appropriate transformations before statistical analysis [6]. The difference of two means between treatments exceeding Critical Difference (CD) value is significant [7].

Results and Discussion
Among the 36 different fractions of plants / plant parts assessed for toxicity against *Fusarium oxysporum*, *Arisaema flavum* roots / tubers hexane fraction recorded lowest radial growth of 5.0 mm followed by its acetone fraction (7.0 mm), *A. flavum* stem + Leaves hexane (7.0 mm) and *Boerrhavia diffusa* roots hexane fraction (7.0 mm), as evident from Table 3. The control plates were completely covered with the mycelia mat of *F. oxysporum*, after seven days (90 mm). Accordingly, the per cent inhibition also varied, being the highest in *A. flavum* roots / tubers hexane (94.44%) followed by its acetone fraction (92.22%), *A. flavum* stem + leaves hexane fraction (92.22%) and *B. diffusa* roots hexane fraction (92.22%). Overall, the hexane fractions of all the plant materials were found more effective in inhibiting *F. oxysporum* radial growth as compared to their acetone and ethanol fractions. This was probably due to more extraction of alkaloids and phytochemicals in hexane fractions. The ethanol fractions were the least effective one.
In accordance with our studies, *a*ntifungal activity of *V. thapsus* was observed in its methanol extract (1000 µg mL-1) against *Fusarium graminearum* and *Macrophomina phaseolina* [8]. *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 obtain the extracts. In the present study, *V. Thapsus* leaves + stem hexane fraction also recorded 76.69% inhibition against *F. oxysporum*. 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 [9]. Likewise, in our study, the *C. grandis* fruits hexane and acetone fraction exhibited 82.22 and 74.44% inhibition respectively. Antibacterial activity of the flowers of *Woodfordia fruticosa* was assessed on different microorganisms [10]. *W. fruticosa* roots hexane fraction showed 77.78% inhibition, showing its antifungal property.
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* [11]. Similarly, the crude extract of *A. flavum* was found active against different bacterial strains (three Gram positive and two Gram negative) [12]. This was in confirmation of our results, wherein *A. flavum* fractions were found very effective in inhibiting the growth of *F. oxysporum*. Similarly, in our study *A. flavum* roots / tubers and stem + leaves hexane, acetone and ethanol fraction exhibited more than 90.0% inhibition against *F. oxysporum*.

Conclusions
These potentially active plant fractions may be further evaluated *in vivo* and utilized for managing plant diseases in the fields. They can also be further developed into botanical pesticides, on 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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Table 3: Fungicidal activity of plant / plant parts fractions

S. No.	Scientific name	Plant part used	Fractions	Radial growth (mm)	Per cent inhibition at 200 ppm
1.	*Achyranthes aspera*	Stem	Hexane	24.0	73.33
2.			Acetone	29.0	67.78
3.			Ethanol	31.0	65.56
4.		Roots	Hexane	23.0	74.44
5.			Acetone	28.0	68.89
6.			Ethanol	31.0	65.56
7.	*Boerhavia diffusa*	Stem	Hexane	8.0	91.11
8.			Acetone	9.0	90.50
9.			Ethanol	12.0	86.67
10.		Roots	Hexane	7.0	92.22
11.			Acetone	8.0	91.00
12.			Ethanol	9.0	90.00
13.	*Murraya Koengii* (L.) sprenge	Fruits	Hexane	23.0	74.44
14.			Acetone	27.0	68.89
15.			Ethanol	31.0	65.56
16.	*Woodfordia fruticosa*	Roots	Hexane	20.0	77.78
17.			Acetone	26.0	71.11
18.			Ethanol	32.0	64.44
19.	*Nicotiana rustica*	Stem + leaves	Hexane	23.0	74.44
20.			Acetone	31.0	65.56
21.			Ethanol	33.0	63.33
22.	*Diplocyclos palmatus*	Fruits	Hexane	25.0	72.22
23.			Acetone	30.0	66.67
24.			Ethanol	34.0	62.22
25.	*Arisaema flavum* (Forsskal) Schott.	Stem + Leaves	Hexane	7.0	92.22
26.			Acetone	8.0	91.00
27.			Ethanol	11.0	87.78
28.		Roots / Tubers	Hexane	5.0	94.44
29.			Acetone	7.0	92.22
30.			Ethanol	9.0	90.00
31.	*Coccinia grandis*	Fruits	Hexane	16.0	82.22
32.			Acetone	23.0	74.44
33.			Ethanol	27.0	68.89
34.	*Verbascum thapsus*	Leaves + Stem	Hexane	21.0	76.69
35.			Acetone	32.0	64.44
36.			Ethanol	35.0	61.11
37.	Control (Carbendazim)			33.0	63.33
38.	Control (Untreated)			90.0	-
S.E. (m)				0.2	2.63
C.D. at 5%				0.54	4.96

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