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Research Article

EVALUATION OF ANTIFUNGAL EFFICIENCY OF 3,7-DIHYDROXY 3′,4′ ORTHODIHYDROXY FLAVONE STUDIED IN RELATION TO SOME MORPHOLOGICAL PARAMETERS IN *PISUM SATIVUM* L. PLANTS

NEELA DAS, PADMA CHATTERJEE*

Department of Botany, Plant Biochemistry, Molecular Biology & Advance Plant Physiology Research Laboratory, University of Kalyani, Kalyani, Nadia - 741 235, West Bengal, India. Email: schatterjeecal2003@yahoo.co.in

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ABSTRACT

Objectives: 3,7-dihydroxy 3′,4′ orthodihydroxy flavone isolated and identified from 50% aqueous ethanolic extract [5] and tested positive in antifungal bioassay against *Fusarium oxysporum ciceri* [6] was taken up for further detailed analysis of the antifungal property for *in vitro* study in relation to *Pisum sativum L.* plants. The study incorporated a comparison of some morphological parameters such as root, shoot, internode, petiole length, leaf and leaflet length, leaf and leaflet number/leaf, the total number of flower/plant, pod/plant, and seeds/pod.

Methods: Seeds of *P. sativum* L. plants were taken up as experimental material for *in vitro* studies. Six different sets were maintained by presoaking the seeds with water, 3,7-dihydroxy 3′,4′ orthodihydroxy flavone, and fungicide for 3 hrs. The seeds were grown in pot cultures. At seedling stage, three sets, one from each treatment, were exposed to fungal inoculum following soil drenching method. At the 21st day, 42nd day, and 63rd day of age, the above-mentioned parameters were studied.

Results: Results indicated increased growth of internode, petiole, and shoot length in the plants which were administered with the 3,7-dihydroxy 3′,4′ orthodihydroxy flavone. The fungus infested plants exhibited reduced growth of internode, petiole, and shoot length. However, griseofulvin, a popularly used fungicide showed inhibition of growth of all the parameters in healthy as well as in the infected plants. Flowering time was delayed in the infested plants. Remarkably again, administration of the plant extract quickened the flowering time as similarly as in the healthy plants. Finally, the number of seeds per pod in the plant also showed the same promising picture. The 3,7-dihydroxy 3′,4′ orthodihydroxy flavone treated plants showed higher number of seeds a pod when administered to the healthy and infected plants.

Keywords: Fungicide, Inhibition, Inoculums, Infestation.

INTRODUCTION

In last few decades, extensive research has been done on various bioactive compounds having antimicrobial effects. From the era of Charak, Sushruta various plants have been used as therapeutic agents.

Different types of measures have been employed for controlling phytopathogenic fungi. The use of synthetic fungicides causes extreme damage to the ecosystem and mankind. These fungi may later become resistant to these compounds [1,2,4]. At this stage, some alternative remedy has been in demand. Antimicrobials of plant origin have enormous therapeutic potentiality and have been used since time immemorial. They have been proved effective in the treatment of infectious diseases simultaneously mitigating many of the side effects which are often associated with synthetic antibiotics [3].

The present work may be cited as an initial platform for a reply to such a demand, where we tried to find out the antimicrobial effect of the leaf extract of *Clitoria ternatea* L. plant against *F. oxysporum ciceri*, if may act as a destructive component of *Pisum sativum* L. plants hindering its productivity. In this study, we depict some morphological parameters of *P. sativum* L. plants during the vegetative, flowering and fruiting stage and try to establish whether the plant extract of *C. ternatea* can be helpful to overcome the damage caused in the *P. sativum* L. plants due to infestation by *F. oxysporum ciceri.*

METHODS

C. ternatea L. plants were collected during the month of April 2014 from the adjoining locality of Kalyani. The foliar parts of the plant materials were sundried, powered, and stored. The *P. sativum* L. seeds of local variety were collected from Bidhan Chandra Krishi Viswavidyala.

Micro-organism used: *F. oxysporum ciceri* collected from the Departmental Stock Culture, Department of Botany, University of Kalyani, Kalyani, Nadia, West Bengal, India. The fungi were grown on PDA medium (pH - 6.8) and incubated at 28°C. Department of Botany, University of Kalyani, Kalyani, Nadia, West Bengal, India.

Preparation of plant extract

The extracted material was dissolved in propylene glycol and used for *in vitro* studies [8].

Seeds of *P. sativum* L. plants were taken up as experimental material for *in vivo* studies. Three different sets were maintained by presoaking the seeds with water, plant extract (applied following minimum inhibitory concentration 60 mg/ml), and fungicide (100 mg/ml) for 3 hrs. Seeds were sown in six sets and plantlets were raised in pot culture method (temperature: 15-18°C, soil pH: 6.5). At the seedling stage, one set from each treatment set was exposed to fungal inoculum following soil drenching method. The plantlets were raised at three stages - vegetative (21st days), flowering (42nd days), and matured (63rd days) stage. Some morphological parameters such as root, shoot, internode, petiole length, leaf and leaflet number/leaf, the total number of flower/plant, pod/plant, and seeds/pod were studied. Data collected in 7 replicates.

RESULTS

Table 1 represents a comparative analysis of some morphological parameters studied on the 21st day. Results indicated that morphological parameters such as the length of root, shoot, petiole, internode, leaf and leaflet were reduced due to fungal infestation. Administration of 3,7-dihydroxy 3′,4′ orthodihydroxy flavone successfully increased all the parameters.

Table 1: Studies on the different morphological parameters of variously treated 21st day old *Pisum sativum* L. plant

Treatment	Shoot length (cm)	Root length (cm) (mean)	Internode length (cm)	Number of leaves/ plant	Leaf length (cm)	Petiole length (cm)	Leaflet length (cm)	Leaflet pair number/ plant	Day of flowering
Healthy	13.64±0.68	12.5±0.12	2.22±0.08	7.71±0.28	2.57±0.07	1.02±0.12	2.66±0.44	2.66±0.21	25
Infested	9.93±0.34	8.85±0.1	1.63±0.14	5.87±0.22	2.7±0.72	0.76±0.11	0.92±0.04	1.40±0.24	32
Infested+compound treated	11.85±0.96	9.78±0.13	1.90±0.10	8.00±0.37	2.77±0.09	1.18±0.09	2.38±0.07	1.81±0.20	25
Compound treated	12.21±0.69	10.45±0.12	1.57±0.13	7.71±0.18	1.80±0.07	1.08±0.08	2.24±0.11	2.60±0.24	28
Fungicide treated	11.78±0.24	8.47±0.2	1.21±0.08	6.85±0.14	1.81±0.08	0.51±0.03	0.88±0.04	1.40±0.24	32
Infested+fungicide treated	10.28±0.69	11.35±0.12	1.95±0.11	6.71±0.18	1.95±0.11	0.58±0.05	1.12±0.04	1.61±0.24	30
LSD	6.49	0.40	0.20	0.95	1.87	0.08	0.37	0.56	-

Calculation was done with the help of statistical software SPSS13.0. LSD: Least significant difference

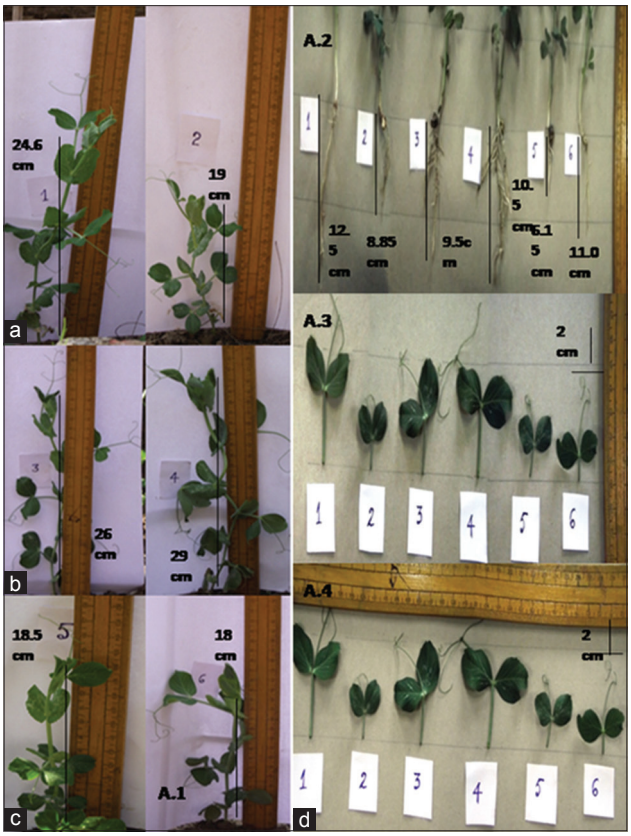

Fig. 1: The vegetative growth state of the plant sets maintained. (a) Shoot length, (b) Root length, (c) Petiole length, (d) Leaflet length. Treatment sets - 1: Healthy, 2: Infested, 3: Infested+compound treated, 4: Compound treated, 5: Fungicide treated, 6: Infested+fungicide treated

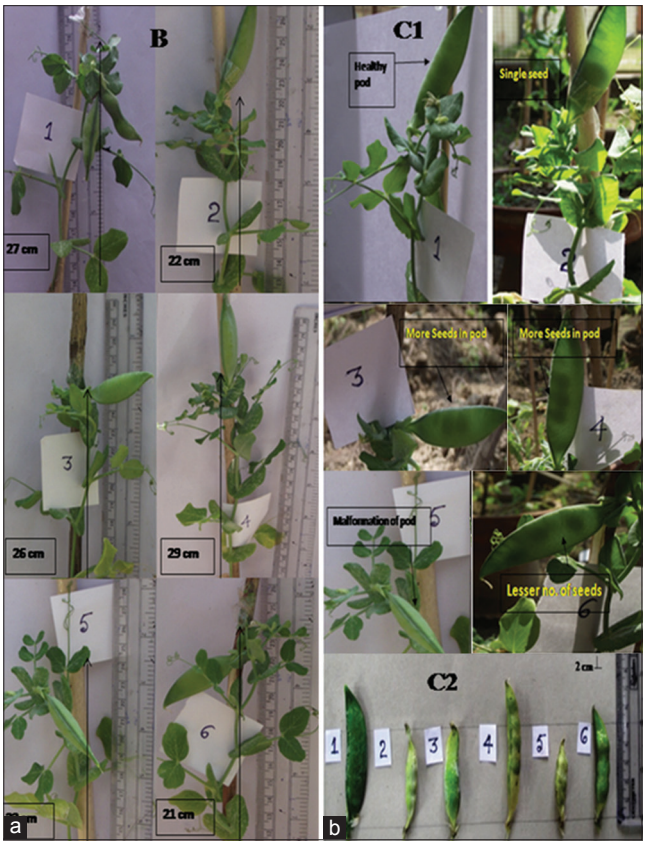

Fig 2: (a) The reproductive state of the plant sets maintained (shoot length) and (b) The pod status of the plant sets maintained. Treatment sets: 1: Healthy, 2: Infested, 3: Infested+compound treated, 4: Compound treated, 5: Fungicide treated, 6: Infested+fungicide treated

Fungus infestation delayed the day of flowering. Administration of the flavone to the fungus infested plants quickened the floral emergence. It was also evident that the promotive effect on the morphological parameters produced by 3,7-dihydroxy 3′,4′ orthodihydroxy flavone were in some cases even higher from that of the healthy plants. These observations are presented in Fig. 1.

Table 2 represents a comparative analysis of some morphological parameters studied on the 42nd day. Results indicated that morphological parameters such as the length of shoot, root, internode, leaf length, leaflet, number of leaves, number of flowers/plant, time of pod emergence (day), number of pods/plants, and number of filled up pods were reduced due to fungal infestation. Administration of 3,7-dihydroxy 3′,4′ orthodihydroxy flavone to the fungus infested plants increased the length of shoot, root, internode, petiole, leaf and leaflet, and hastened the time of pod emergence. These results are documented in Fig. 2a.

Table 3 represents a comparative analysis of some morphological parameters studied on the 42nd day Results indicated that fungal infestation reduced the length of root, shoot, petiole, internode, leaf, and leaflet and were caused deformities and malformation in pod and reduced number of seeds/pod. Administration of the 3,7-dihydroxy 3′,4′ orthodihydroxy flavone to the fungus infested plants increased the length of shoot, root, internode, leaf length, leaflet, number of leaves and produced round healthy pods with increased no of seeds. All these parameters can be supported by Fig. 2b.

DISCUSSIONS

While comparing the antifungal activity of the herbal 3,7-dihydroxy 3′,4′ orthodihydroxy flavone with that of gresiofulvin, it was also inferred that gresiofulvin was less active as an antifungal agent during the study and also toxic to the healthy plants. Whereas, the

Table 2: Studies on the different morphological parameters of variously treated 42nd day old *Pisum sativum* L. plant

Treatment	Shoot length (cm)	Root length (cm)	Internode length (cm)	Leaves number	Leaf length (cm)	Leaflet length (cm)	Number of flower/ plant	Pod emergence (day)	Number of pods/ plants	Pod number with seed
Healthy	22.2±0.66	11.05±14	2.68±0.14	7.6±0.24	2.68±0.11	3.55±0.18	1.33±0.21	28th	1.50±0.22	All
Infested	17.2±1.01	8.5±0.03	1.4±0.04	5.0±0.36	2.04±0.22	1.34±0.06	0.50±0.22	36th	1.10±0.16	All
Infested+compound treated	23.2±0.37	11.2±0.8	2.83±0.21	8.83±0.3	2.53±0.09	3.88±0.03	2.60±0.24	34th	1.80±0.20	All
Compound treated	22.7±1.64	12.45±0.15	3.01±0.19	10.1±0.34	2.37±0.12	2.30±0.12	1.80±0.20	34th	2.0±0.20	All
Fungicide treated	11.66±0.33	8.17±	1.32±0.09	6.66±0.21	1.18±0.07	1.36±0.06	0	40th	0	All
Infested+fungicide treated	13.58±0.20	10.65±0.19	1.9±0.01	3.60±0.24	1.70±0.12	1.73±0.08	0	0	0	None
LSD	8.98	0.2	0.267	1.08	0.1845	0.082	0.564	0	0.326	None

Calculation was done with the help of statistical software SPSS13.0. LSD: Least significant difference

Table 3: Studies on the different morphological parameters of variously treated 63rd day old *Pisum sativum* L. plant

Treatment	Root length (cm)	Shoot length (cm)	Inter node length (cm)	Leaf length (cm)	Petiole length (cm)	Leaflet length (cm)	Details of pod morphology			Seed morphology	
							Shape	Size	Seed/pod	Shape	Growth state
Healthy	12.5±0.10	24.64±0.14	2.98±0.08	4.5±0.08	2.75±0.07	2.4±0.08	Regular	6.9±0.08	4.30±0.18	Normal	Healthy normal
Infested	9.85±0.09	19.37±0.07	1.98±0.08	2.48±0.08	2.15±0.14	1.71±0.07	Small curved	5.4±0.08	2.0±0.18	Small	Maldeveloped
Infested+ compound treated	10.78±0.10	26.61±0.13	3.44±0.07	2.97±0.08	3.14±0.12	2.6±0.11	Regular normal	6.9±0.08	4.30±0.18	Normal	Healthy
Extract treated	8.45±0.07	34.65±0.14	4.02±0.12	3.48±0.08	3.52±0.10	3.0±0.09	Regular normal	7.0±0.09	4.30±0.18	Normal	Healthy
Fungicide treated	8.47±0.08	18.45±0.07	2.44±0.07	2.48±0.08	2.5±0.08	2.48±0.08	Curved wrinkle small	4.9±0.08	2.30±0.18	Small	Immatured
Infested+ fungicide treated	11.35±0.09	18.42±0.08	1.92±0.07	1.98±0.08	2.46±0.11	1.97±0.08	Small, wrinkled	4.9±0.08	0	-	-
LSD	0.121	0.161	0.101	0.101	0.161	0.101		0.10	0.21		

LSD: Least significant difference

leaf extract exhibited a better antifungal property with no toxic effect.

The extent of positive effect shown by the 3,7-dihydroxy 3′,4′ orthodihydroxy flavone was in some cases even more from that of healthy or in the rest more or less similar to than that of healthy. Hence, the findings suggest that the 3,7-dihydroxy 3′,4′ orthodihydroxy flavone of *C. ternatea* L. not only possess a non-hazardous antifungal property but also may be formulated as a source of growth accelerator at a certain stage of growth and development.

CONCLUSION

From the study undertaken it may be clearly concluded that the leaf extract of *C. ternatea* L. not only acted as antifungal agent but also promoted some of the growth and developmental parameters of *P. sativum* L. plants over healthy, such as shoot length, root length, petiole length, number of flowers/plant, and pod length.

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