Research Article

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ANTIFUNGAL EFFECT OF *CLITORIA TERNATEA* L. LEAF EXTRACT ON SEEDS OF *PISUM SATIVUM* L. IN RELATION TO THE ACTIVITIES OF SOME ENZYMES

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ABSTRACT

This paper highlights the antifungal activities of the leaf extract of *Clitoria ternatea* L. against *Fusarium oxysporum ciceri.* The experimental procedures include study of the activities of some marker enzymes of seeds of *Pisum sativum* L. The leaf extract of *Clitoria* showed antifungal activity in relation to some enzyme activities. Fungus infestation increased activity level of some stress enzymes like catalase, peroxidase and Superoxide Dismutase which was controlled by the administration of the plant extract. Furthermore the activities of amylase, protease and dehydrogenase were decreased by the fungal infestation which was overcome by application of the plant extract. The findings of the work thus supports that the formulation can be used as an antifungal agent against the fungus to overcome the deleterious effect caused by it on the pea seeds.

Keywords: antifungal, antimicrobial formulation, stress, enzyme activity, infestation

INTRODUCTION

In last few decades, extensive research has been done to find out 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 those compounds. At this stage, some alternative remedies are in demand. Some of the traditional medicine involves the use of crude plant extracts, which may contain an extensive diversity of molecules, often with indefinite biological effects[1]. *C. ternatea* L. commonly known as Butterfly pea belonging to the family Fabaceae and sub-family Papilionaceae is a perennial leguminous twinner, which originated from tropical Asia and later was distributed widely in South and Central America, East and West Indies, China and India, where it has become naturalised. It is also commonly called as *Clitoria*, blue-pea, kordofan pea (Sudan), cunha (Brazil or pokindong (Philippines) and is a vigorous, summer growing, legume of old world origin. The most frequently reported species is *C. ternatea* L. The major phytoconstituents found in the plant are the pentacyclic triterpenoids such as taraxerol and taraxerone[2,3]. The seeds contain nucleoprotein with its amino-acid sequence similar to insulin, delphinidin-3,3,5-triglucoside, essential amino-acids, pentosan, water soluble mucilage, adenosine, an anthoxanthin glucoside, greenish yellow fixed oil[4,5] a phenol glycoside, 3,5,7,4-tetrahydroxy-flavone-3-rhamoglycoside, an alkaloid, ethyl D-galactopyranoside, p-hydroxy cinnamic acid polypeptide, a highly basic protein-finotin, a bitter acid resin, tannic acid, 6 % ash and a toxic alkaloid. According to Yoganarasimhan seeds contain ß-sitosterol, and hexacosanol and anthocyanin glucoside[6-9]. It also contains anti-fungal proteins and has been shown to be homologous to plant defensins[10]. Since the purified lectin was found to be potential tool for cancer studies so an attempt was made for the alternate high yielding purification method for *C. ternatea* lectin designated CTL, present in the seeds of this member of leguminosae family[11,12]. The present work may be cited as an initial platform for a reply to such a demand, where we tried to find out the anti microbial effect of the leaf extract of *Clitoria ternatea* L. plant against *F. oxysporum ciceri* that acts as a destructive pathogen of *Pisum sativum* L. plants hindering its productivity. In this paper we tried to establish the antifungal efficiency of 50 % aqueous extract of *Clitoria ternatea* L. against *Fusarium oxysporum ciceri* in *Pisum sativum* L. seeds in relation to the activities of some marker enzymes of metabolism. The study also entailed isolation of a purified bioactive sample and its antifungal potentiality in terms of the studied enzymes.

MATERIALS AND METHODS

Clitoria ternatea L. plants were collected. The foliar parts of the plant materials were dried, powered and stored. The *Pisum sativum* L. seeds of local variety were collected from Bidhan Chandra Krishi Visvavidyala. Micro-organism used: *Fusarium oxysporum ciceri* collected from the departmental stock culture, Department of Botany, University of Kalyani, Kalyani, Nadia, West Bengal, India. The fungi was grown on PDA medium (pH- 6.8) and incubated at 28°C, Department of Botany, University of Kalyani.

Preparation of plant extract

100 g of dried powered leaves of *Clitoria ternatea* L. were sequentially soaked in petroleum ether (60-80^{0}c), chloroform and 50 % aqueous ethanol for seven days. The extracts were collected separately, evaporated under reduced pressure in a vacuum rotary evaporator. Dark green and brown residual solids collected from each fraction were subjected to agar cup diffusion bioassay method[13] against *Fusarium oxysporum ciceri*. Activity was located in 50 % aqueous ethanolic fraction. Seeds of *Pisum sativum* L. plants were taken up as experimental material for *in vitro* studies. Six different sets were maintained by presoaking the seeds with water, plant extract (applied following MIC 60 mg/ml) and fungicide (100

mg/ml) for 3 hours. After that half of the seeds from each set were exposed to fungal inoculum.

Determination of enzyme activities

The enzyme activities were calculated by the Fick and Qualset, (1975) formula[14]:

Enzyme activity= Δ O.D.* tv / T * v * w
Where, Δ OD = difference in OD. Values, tv = total volume of extract, v = volume of enzyme taken for reaction, w = weight of tissue used, T = total time

Following enzymes were studied:

- Amylase: Khan and Faust, 1967[15],
- Catalase: Snell and Snell, 1971[16], modified by Biswas and Choudhuri, 1978[17],
- Dehydrogenase: Rudrapaul and Basu, 1979[18],
- Peroxidase: Chance and Maehly, 1955[19], modified by Kar and Mishra, 1976[20],
- Protease: Biswas and Choudhury, 1978[21],
- Superoxide Dismutase (SOD): Method of Asada *et al*.1974[22]

Table 1: General screening of petroleum ether, chloroform and 50 % aqueous ethanolic extract against *Fusarium oxysporum ciceri*

Clitoria ternatea L.	Diameter of inhibition zone (cm)	Inhibition percentage (%)
Petroleum ether	–	-
Chloroform	–	-
50 % ethanol	0.5	17.0

Table 2: Studies on the effect of 50 % aq. ethanolic extract and bioactive sample on amylase and protease activity in *Pisum sativum* L. seeds

Treatment sets	Amylase activity			Protease activity		
	after 24 h	after 48 h	after 72 h	after 24 h	after 48 h	after 72 h
Healthy	5054.8 ± 31.8	3235.4 ± 16.1	2082.9 ± 13.0	942.484 ± 7.217	718.265 ± 7.257	599.684 ± 5.782
Fungus infested	4786.0 ± 140.8	1710.8 ± 17.3	1170.4 ± 8.7	600.517 ± 5.797	609.959 ± 2.887	439.785 ± 2.895
Fungus infested + 60 mg / ml F	4901.7 ± 13.0	3313.8 ± 13.1	2815.2 ± 8.7	714.738 ± 5.779	541.237 ± 74.048	517.550 ± 4.330
Healthy + 60 mg/ml F	6454.8 ± 29.3	3017.7 ± 13.0	2975.2 ± 14.4	725.135 ± 4.330	625.817 ± 4.183	602.416 ± 10.104
Healthy gresiofulvin treated	6029.9 ± 25.0	5056.8 ± 47.5	4595.0 ± 20.0	240.289 ± 2.901	72.357 ± 1.450	209.166 ± 2.619
Fungus infested + gresiofulvin treated	4947.1 ± 45.1	3644.3 ± 22.7	2150.0 ± 15.0	565.314 ± 2.904	129.792 ± 2.042	448.220 ± 4.390

Table 3: Studies on the effect of 50 % aq. ethanolic extract and bioactive sample on dehydrogenase and peroxidase activity in *Pisum sativum* L. seeds

Treatment sets	Dehydrogenase activity			Peroxidase activity		
	after 24 h	after 48 h	after 72 h	after 24 h	after 48 h	after 72 h
Healthy	88.790 ± 0.680	461.937 ± 1.488	148.460 ± 0.680	0.497 ± 0.012	1.417 ± 0.083	2.384 ± 0.113
Fungus infested	52.803 ± 0.921	339.793 ± 1.244	118.993 ± 0.639	0.753 ± 0.030	2.245 ± 0.036	2.542 ± 0.114
Fungus infested + 60 mg/ml F	140.686 ± 2.442	607.777 ± 1.841	155.185 ± 1.152	0.575 ± 0.015	1.306 ± 0.118	1.811 ± 0.087
Healthy + 60mg/ml F	63.023 ± 0.563	472.510 ± 1.127	131.843 ± 0.865	0.154 ± 0.035	1.347 ± 0.142	1.766 ± 0.118
Healthy + gresiofulvin treated	34.707 ± 0.859	302.797 ± 1.455	101.447 ± 0.866	0.609 ± 0.026	1.615 ± 0.087	1.698 ± 0.085
Fungus infested + gresiofulvin treated	31.677 ± 2.084	408.707 ± 1.429	96.703 ± 0.895	0.588 ± 0.057	1.602 ± 0.005	1.650 ± 0.089

Table 4: Studies on the effect of 50 % aq. ethanolic extract and bioactive sample on catalase super oxide dismutase activity in *Pisum sativum* L. seeds

Treatment sets	Catalase activity			Super Oxide Dismutase activity		
	after 24 h	after 48 h	after 72 h	after 24 h	after 48 h	after 72 h
Healthy	0.183 ± 0.004	0.232 ± 0.019	0.073 ± 0.007	1006.663 ± 8.135	1066.00 ± 3.786	872.667 ± 2.728
Fungus infested	0.242 ± 0.037	0.323 ± 0.018	0.083 ± 0.007	1081.667 ± 3.087	1199.667 ± 3.087	1229.667 ± 3.919
Fungus infested + 60 mg/ml F	0.305 ± 0.008	0.142 ± 0.024	0.057 ± 0.018	894.000 ± 3.253	1254.000 ± 4.770	1004.633 ± 3.435
Healthy + 60 mg/ml F	0.247 ± 0.026	0.040 ± 0.012	0.075 ± 0.009	993.327 ± 2.721	1304.333 ± 4.586	901.330 ± 2.960
Healthy + gresiofulvin treated	0.318 ± 0.028	0.097 ± 0.015	0.130 ± 0.035	811.663 ± 2.390	1163.997 ± 2.954	1333.300 ± 4.073
Fungus infested + gresiofulvin treated	0.272 ± 0.006	0.040 ± 0.012	0.080 ± 0.012	764.833 ± 2.024	979.000 ± 2.179	981.330 ± 4.203

RESULTS AND DISCUSSION

Table 1 represent general screening of petroleum ether, chloroform and 50 % aqueous ethanolic extract against the fungus selected. Result revealed 50 % aqueous ethanolic extract was found to be active against the fungus. From Table 2 and 3, it could be inferred that infestation of *Pisum sativum* seeds with *Fusarium oxysporum* caused reduction in the activities of amylase, protease and dehydrogenase enzymes. Administration of 50 % aqueous ethanolic extract of *Clitoria* increased the enzyme activities appreciably leading towards control. In all the three cases, the extract and the purified sample showed higher potentiality than that off gresiofulvin. Table 3 and 4 depicts the antifungal effect of 50 % aqueous ethanolic extract in relation to the activities of the stress enzymes catalase, peroxidase and superoxide dismutase. Results in all the three cases indicated sharp rise in the enzyme activities upon fungal infestation of *Pisum* seeds by *Fusarium oxysporum*. Presoaking the seeds with 50 % aqueous ethanolic extract of *Clitoria* and the purified bioactive sample retarded the activities of the stress enzymes. The antifungal efficiency of the leaf extract and purified sample in terms of antifungal activity was also higher than gresiofulvin.

CONCLUSION

Hence from the above results it can be inferred that the crude 50 % aqueous leaf extract of *Clitoria ternatea* L. showed antifungal activity in relation to the activities of the studied

enzymes. Fungal infestation resulted in decrease in amylase, protease and dehydrogenase activity in the respective sets which were overcome in the extract treated sets. In the same way, fungal infestation induced increased activity of the stress enzymes were lowered by the extract treatment. The extract helped to maintain the normal activity level of the enzymes in the fungus infested seed sets compared to that of the control sets. So it can be said that *C. ternatea* L. may be used as a crop protectant of *Pisum sativum* seeds against *Fusarium oxysporum* infestation.

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