Research Article

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ANTIFUNGAL EFFECT OF *CLITORIA TERNATEA* L. LEAF EXTRACT ON SEEDS OF *PISUM SATIVUM* L. IN RELATION TO SOME BIOCHEMICAL PARAMETERS

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ABSTRACT

During the last few decades raising of anti-hazardous eco-friendly formulation for therapeutic purposes has gained immense importance. Hence the present work was taken to investigate any bio control measure in *Clitoria ternatea* L. leaf extract in relation to protecting *Pisum sativum* L. seeds against *Fusarium oxysporum* attack. Different types of extracts of *Clitoria ternatea* (viz. petroleum ether, chloroform and 50 % ethanolic extracts) was tested against *Fusarium oxysporium* for its antifungal activity by agar cup method. Active fraction was also tested on *Pisum* seeds for its efficiency to protecting seeds against infection caused by *F. oxysporium*. The experiments include estimation of few physiological and biochemical parameters like sugar, amino acid, protein, proline, phenol and nucleic acid in the healthy and *Fusarium oxysporum* infested seeds of *Pisum sativum* L. The 50 % ethanolic leaf extract of *Clitoria* showed antifungal activity against *Fusarium oxysporum ciceri* and extract treatment exhibited increased level of biochemical parameters compared to that of fungus infested seed sets. The study revealed that the change in the levels of sugar, amino acid, protein, proline, phenol and nucleic acid caused by the fungal infestation was protected by treatment of the infested seeds by administration of the test sample *C. ternatea* L. by presoaking method. From this study it may infer that the 50 % ethanolic leaf extract of *Clitoria ternatea* L. has antifungal activities and it can be used to protect the *Pisum sativum* seed from the attack of *Fusarium oxysporium*.

Keywords: Stress, phytopathogen, bio control measures.

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 phyto-pathogenic 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 medicines involve the use of crude plant extracts, which may contain an extensive diversity of molecules, often with indefinite biological effects[1]. Different types of measures have been employed for controlling phytopathogenic fungi. At this stage, some alternative remedy has been in demand. 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 *C. ternatea* L. plant against *F.oxysporum ciceri*; that acts as a destructive component of *P. sativum* L. plants hindering its productivity. *Clitoria 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 naturalized. 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 *Clitoria ternatea*. The major phyto-constituents 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 phenolglycoside 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[6-8]. It also contains anti-fungal proteins and has been shown to be homologous to plant defensins[9]. 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[10,11]. In this paper we tried to establish the antifungal efficiency of 50 % aqueous leaf extract of *C. ternatea* L. against *F. oxysporum ciceri* in *P. sativum* L. seeds in relation to few physiological and biochemical parameters.

MATERIAL AND METHOD

Clitoria ternatea L. plants were collected during the month of April 2013 from the adjoining locality of Kalyani, India. The foliar parts of the plant materials were sundried, powdered and stored. The *P. sativum* L. seeds of local variety were collected from Bidhan Chandra Krishi Viswa Vidyalaya. 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 was grown on PDA medium (pH-6.8) and incubated at 28°C at Department of Botany, University of Kalyani, India.

Preparation of plant extract

100 g of dried powdered leaves of *C. ternatea* L. were sequentially soaked in petroleum ether (60-80°), chloroform and 50 % aqueous ethanol for three days each. The extracts were collected separately, reduced in high pressure vacuum rotary evaporator. Dark green and brown residual solids collected from each fraction were subjected to agar cup diffusion bioassay method[12] against *F. oxysporum ciceri*. Activity was located in 50 % aqueous ethanolic fraction. The residual solid obtained from concentrating 50 % aqueous ethanolic fraction was taken as the test sample. Seeds of *P. sativum* L. plants were taken up as experimental material for *in vitro* studies. Three different sets were maintained by presoaking the seeds with water, plant extract (applied following MIC 60 mg/ml) of the test sample dissolved in propylene glycol and fungicide (100 mg/ml) for 3 hours. After that half of the seeds from each set were exposed to fungal inoculum. At interval of 24, 48 and 72 h; the following experiments were carried out to estimate the biochemical parameters:

- Determination of total soluble sugar content[13],
- Determination of Protein content[14],
- Determination of Amino acid content[15,16],
- Determination of phenol content[17],
- Estimation of o-dihydric phenols[18],
- Extraction and estimation of total proline[18],
- Extraction and determination of nucleic acid content[19-22].

Table 1: Screening of various fractions of *C. ternatea* L. against *Fusarium oxysporum ciceri*

Fractions of *Clitoria ternatea* L.	Dia. of inhibition zone (cm)	Inhibition percentage (%)	MIC (mg/ml)
Petroleum ether		-	-
Chloroform		-	-
50%ethanol	0.5	37.0	60

Values in the table indicates the mean values

Table 2: Studies on the effect of 50 % Aq. ethanolic extract on total sugar and protein content in *Pisum sativum* L. seeds

Treatment sets	Total sugar content (mg/g) after			Total protein content (mg./g.) after		
	24 h	48 h	72 h	24 h	48 h	72 h
Healthy	20.198 ± 0.94	39.438 ± 0.4	30.307 ± 1.0	68.820 ± 5.387	64.302 ± 3.173	83.134 ± 6.114
Fungus infested	11.021 ± 1.059	20.100 ± 0.5	12.366 ± 1.901	42.418 ± 4.063	47.609 ± 4.771	80.709 ± 7.029
Fungus infested + 60 mg/mlF	82.872 ± 2.00	97.74 ± 2.503	20.257 ± 0.401	41.628 ± 4.887	49.279 ± 4.005	75.675 ± 3.692
Healthy + 60 mg/mlF	46.688 ± 0.3	49.828 ± 0.751	32.014 ± 2.0	45.507 ± 9.232	61.949 ± 7.089	95.685 ± 4.791
Healthy + gresiofulvin treated	65.693 ± 2.0	66.711 ± 0.6	40.448 ± 0.5	48.657 ± 5.165	39.765 ± 5.001	78.155 ± 5.165
Fungus infested + gresiofulvin treated	63.410 ± 7.518	61.890 ± 5.558	25.377 ± 0.402	38.598 ± 7.084	56.960 ± 5.738	80.051 ± 3.944

Values in the tables indicates the mean value ± standard deviation

Table 3: Studies on the effect of 50 % Aq. ethanolic extract on total amino acid and phenol content in *Pisum sativum* L. seeds

Treatment sets	Total amino acid content (µg/g) after			Total phenol content (µg/g) after		
	24 h	48 h	72 h	24 h	48 h	72 h
Healthy	408.398 ± 7.003	498.437 ± 12.002	470.925 ± 19.0	31.957 ± 1.8	70.359 ± 2.6	65.966 ± 3.350
Fungus infested	345.895 ± 5.0	352.356 ± 3.007	292.632 ± 17.001	49.473 ± 1.9	94.675 ± 2.471	80.436 ± 2.2
Fungus infested + 60 mg/mlF	379.157 ± 8.502	849.572 ± 45.006	346.986 ± 10.007	30.837 ± 1.354	64.780 ± 1.352	40.718 ± 1.372
Healthy + 60 mg/mlF	983.504 ± 21.001	1223.653 ± 24.008	11000.390 ± 15.5	45.824 ± 2.350	55.619 ± 1.3	65.187 ± 2.1
Healthy + gresiofulvin treated	204.052 ± 10.014	388.596 ± 11.002	303.027 ± 9.0	43.149 ± 1.350	56.160 ± 1.251	188.303 ± 7.051
Fungus infested + gresiofulvin treated	558.259 ± 30.0	590.701 ± 13.507	508.352 ± 12.001	62.561 ± 2.0	155.156 ± 1.910	127.324 ± 5.402

Values in the tables indicates the mean value ± standard deviation

Table 4: Studies on the effect of 50 % Aq. ethanolic extract on total DNA and RNA content in *Pisum sativum* L. seeds

Treatment sets	Total DNA (µg/g) content after			Total RNA content (µg/g) after		
	24 h	48 h	72 h	24 h	48 h	72 h
Healthy	196.925 ± 4.035	188.295 ± 6.002	271.698 ± 5.016	17.31837 ± 0.356	24.051 ± 0.491	27.236 ± 0.425
Fungus infested	183.320 ± 2.008	173.291 ± 5.002	251.974 ± 11.014	10.487 ± 0.317	21.085 ± 0.801	21.770 ± 0.378
Fungus infested + 60 mg/mlF	188.997 ± 5.000	242.813 ± 3.036	275.402 ± 10.002	12.727 ± 0.180	16.255 ± 0.625	24.671 ± 0.302
Healthy + 60 mg/mlF	204.960 ± 5.0	262.594 ± 7.500	289.375 ± 9.002	10.718 ± 0.346	14.821 ± 0.825	29.512 ± 0.700
Healthy + gresiofulvintreated	196.551 ± 5.079	242.334 ± 6.049	297.438 ± 8.003	10.423 ± 0.495	11.607 ± 0.495	37.974 ± 1.150
Fungus infested + gresiofulvin treated	256.803 ± 4.026	258.463 ± 8.004	296.755 ± 9.010	11.241 ± 0.490	25.135 ± 0.910	34.485 ± 0.402

Values in the tables indicates the mean value ± standard deviation

Table 5: Studies on the effect of 50 % Aq. ethanolic extract on total Ortho-dihydric phenol and proline content in *Pisum sativum* L. seeds

Treatment sets	Total O-dihydric phenol content (µg/g) after			Total proline content (µg/g) after		
	24 h	48 h	72 h	24 h	48 h	72 h
Healthy	200.0 ± 9.00	118.796 ± 14.170	102.030 ± 12.528	134.804 ± 2.506	98.718 ± 1.750	70.246 ± 0.947
Fungus infested	181.954 ± 8.504	120.3 ± 14.507	78.496 ± 8.5	249.423 ± 5.796	199.184 ± 4.503	96.250 ± 1.059
Fungus infested + 60 mg/ml F	192.481 ± 10.016	103.759 ± 7.535	96.015 ± 8.623	83.285 ± 2.20	134.023 ± 3.252	73.130 ± 2.0
Healthy + 60 mg/mlF	193.984 ± 15.265	102.255 ± 3.527	90.496 ± 8.005	87.349 ± 2.501	93.416 ± 1.701	85.034 ± 0.3
Healthy + gresiofulvin treated	177.443 ± 12.314	112.781 ± 8.012	99.248 ± 7.501	329.623 ± 9.035	286.324 ± 7.01	99.422 ± 2.007
Fungus infested + gresiofulvin treated	190.977 ± 15.178	111.278 ± 10.509	112.781 ± 7.501	361.143 ± 4.515	99.362 ± 2.501	67.161 ± 7.518

Values in the tables indicates the mean value ± standard deviation

RESULTS AND DISCUSSION

Results of Table 1 showed that the 50 % ethanolic leaf extract of the plant exhibited the antifungal property against *Fusarium oxysporum ciceri* showing the MIC value of 60 mg/ml. Table 2 exhibited the test results of sugar and protein contents of the seed treatment sets. The sugar content was decreased in the fungus infested sets as compared to the control set whereas the application of the test sample increased the level in both in the fungus infested as well as in healthy sets. Same observation can be seen in the protein level also. Fungus infestation caused a decrease in the protein level in all the sets and administration of the bioactive sample increased the level in the extract treated sets. The results of total amino acid and phenol content were exhibited in Table 3. Total amino acid content in all the six treatment sets were increased in the 48 h treatment set. The content was decreased in the fungus infested sets as compared to the control set whereas the application of the test sample increased the level in both in the fungus infested as well in healthy sets. Same observation could also be seen in the total phenol level but fungus infestation caused an increase in the phenol level in all the sets and administration of the bioactive sample decreased the level in healthy sets. Table 4 exhibited the results of total DNA and RNA content in all the treatment sets. The fungus infestation decreased the content level in all the sets compared to that of the control set but the administration of the bioactive sample rectified the content level in the fungus infested sets. Table 5 exhibited the results of total or the o-dihydric phenol and proline contents of the treatment sets. The fungus infestation decreased the content level in all the sets compared to that of the control set but the administration of the bioactive sample rectified the content level in the fungus infested sets. Fungus infestation caused an increase in the proline level in all the sets and administration of the bioactive sample decreased the level in the test sample treated sets. The same observation found in the phenol test was exhibited in the o-dihydric phenol test.

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 some biochemical parameters. Fungal infestation resulted in increase in proline, o-dihydric phenol and phenol content which was decreased by bioactive sample treatment. The extract treatment increased sugar, protein and amino acid content in the healthy sets. The extract helped to improve the levels of the quantitative parameters in the fungus infested seeds over the healthy. So it can be said that the damage caused by fungal infestation could be rectified by treatment of the infested seeds by administration of the test sample from *C. ternatea* L. by presoaking method.

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