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RESEARCH ARTICLE

ANTIMICROBIAL ACTION OF 2-PYRROLIDINONE IDENTIFIED FROM *NEPHILA ANTIPODIANA*

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ABSTRACT

Spider's web from *Nephila antipodiana* has been identified for producing a significant repellant activity against pests and ants. In order to investigate the repellant activity, screening of various compounds in the web was performed by GC-MS. An alkaloid namely, 2-pyrrolidinone was observed to act as a predator deterrent in species of moths, caterpillars and ants. For analyzing the feasibility of this alkaloid as an anti microbial action, antimicrobial studies were performed. The evaluation was done by well diffusion assay on two species of bacteria and fungi plant pathogens. Different concentration of 2-pyrrolidinone were loaded into the wells and the zone of inhibition was measured. Inhibition curves were compared, to check the effect of 2-pyrrolidinone between the bacterial and fungal species.

INTRODUCTION

Agriculture has manifested to be the reason for the economic growth of farmers in India. In our present agricultural attic fertilizers are applied to supply essential nutrients. The use of pesticides has improved the agricultural yield but also produces harmful effects to the environment and humans. An alternate to chemical pesticides are biopesticides which encompass a broad array of microbial pesticides, biochemicals derived from micro organisms and other genetically modified agricultural commodities that confer protection against pest damage. One such compound present in the silk of the spider was found to act as a deterrent. The compound repels ant attack from an alkaloid present in the web of a golden orb spider, commonly known as Batik golden orb spider or *Nephila antipodiana*. The impressive properties of spider silk already known to be very strong, elastic, adhesive and may provide new opportunities for pesticide design.

So our present work focuses with three important things. Primarily, screening of spiders web for the identification of naturally occurring biocontrol compound. Secondly, to evaluate the antibacterial and antifungal activities against various plant pathogens. Lastly, comparing the inhibition curves for both the bacterial species by evaluating their zone of inhibition.

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MATERIALS AND METHODS

Screening and identification of biocontrol compound from the web of Nephila antipodiana

The screening of the spider's web for the presence of a biocontrol compound was done by GC-MS. 2.5 mg of the spider's web was taken and dissolved with 1 ml of HCl. Then 1 ml of methanol was added to the above solution. Solvent extraction was done by shaking the vial with the silk for 10 minutes. The extract was concentrated by blowing nitrogen gas until the amount of extract was 10 l. then 2 µl of the extract was inserted into GC-MS-QP2010 (SHIMADZU) system. The compounds were isolated and identified.

Antimicrobial studies of 2-pyrrolidinone against different bacterial and fungal species

Antibacterial activity of 2-pyrrolidinone against two bacterial pathogens namely, *Pseudomonas syringae* and *Xanthomonas campestris* was done by well diffusion assay. Nutrient agar was prepared, sterilized and poured in the sterile petridishes. 24 hour growing culture of the both the bacterial species were swabbed onto solidifies agar medium in different petriplates. The wells were made by using a pasteur pipette. 50 µl of different concentrations (0.064, 0.128, 0.192, 0.256, 0.320, 0.384, 0.448, 0.512, 0.576, 0.640 mg) of 2-pyrrolidinone were loaded into the wells. The plates were incubated at 37 ºC for 24 hours and the zone of inhibition was measured. Antifungal activity of 2-pyrrolidinone against two fungal pathogens namely, *Curvularia lenata and Fusarium oxysporum* was done

by well diffusion assay. Potato dextrose agar was prepared, sterilized and poured in the sterile petridishes. 24 hour growing culture of the both the bacterial species were swabbed onto solidifies agar medium in different petriplates. The wells were made by using a pasteur pipette. 50 µl of different concentrations (0.064, 0.128, 0.192, 0.256, 0.320, 0.384, 0.448, 0.512, 0.576, 0.640 mg) of 2-pyrrolidinone were loaded into the wells. The plates were incubated at 37 ºC for 24 hours and the zone of inhibition was measured.

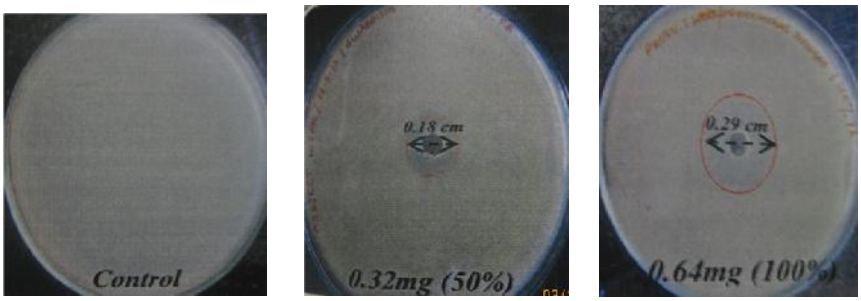
RESULTS

The components present in the spiders silk were identifies by GC-MS-QP2010 (SHIMADZU). Various organic compounds present in the sample were identified using this technique and presented in the form of a chromatogram.

The pyrrolidine alkaloid (2-pyrrolidinone) was found to be present in the spider's web. The bacterial and the fungal strain were swabbed on the nutrient agar and potato dextrose agar respectively. Wells were made using Pasteur pipette on each plate and different concentrations (0.064, 0.128, 0.192, 0.256, 0.320, 0.384, 0.448, 0.512, 0.576, 0.640 mg) of 2-pyrrolidinone were loaded into the wells. The plates were incubated at 37 ºC for 24 hours and the zone of inhibition was measured and listed in Table 1 and table 2 and their images with 50% and 100% concentrations were shown in Figure 1, 2, 3 and 4.

Table 1. Antibacterial activity of 2-pyrrolidinone against bacterial plant pathogens

Bacterial plant pathogen	2-pyrrolidinone concentration (mg)									
	0.064	0.128	0.192	0.256	0.320	0.384	0.448	0.512	0.576	0.640
P. syringae	0.00	0.08	0.10	0.12	0.18	0.21	0.22	0.24	0.26	0.29
X. campestris	0.00	0.04	0.06	0.10	0.11	0.12	0.15	0.16	0.17	0.19

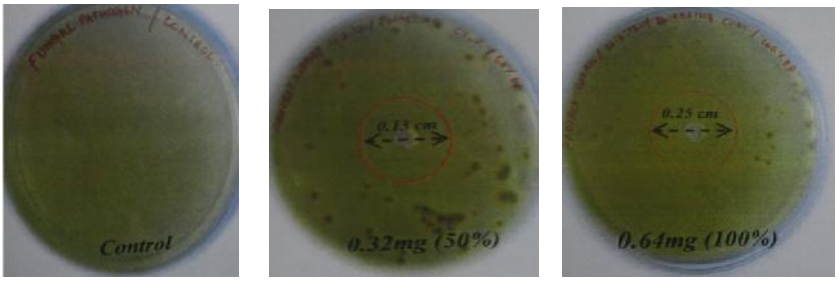


a **b** **c**

Fig. 1a represents the control petriplate with nutrient agar, Fig. 1b represents the 0.32 mg concentration of 2-pyrrolidinone against *P. syringae* which shows a zone of inhibition of 0.18 cm and Fig. 1c represents the 0.64 mg concentration of 2-pyrrolidinone against *P. syringae* which shows a zone of inhibition of 0.29 cm

a **b** **c**

Fig. 2a represents the control petriplate with nutrient agar, Fig. 2b represents the 0.32 mg concentration of 2-pyrrolidinone against *X. campestris* which shows a zone of inhibition of 0.11 cm and Fig. 2c represents the 0.64 mg concentration of 2-pyrrolidinone against *X. campestris* which shows a zone of inhibition of 0.19 cm

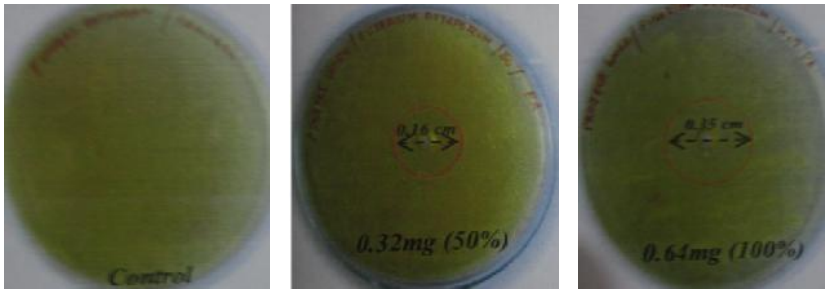


a **b** **c**

Fig. 3a represents the control petriplate with potato dextrose agar, Fig. 3b represents the 0.32 mg concentration of 2-pyrrolidinone against *C. lenata* which shows a zone of inhibition of 0.13 cm and Fig. 3c represents the 0.64 mg concentration of 2-pyrrolidinone against *C. lenata* which shows a zone of inhibition of 0.25 cm

Table 2. Antifungal activity of 2-pyrrolidinone against funal plant pathogens

Fungal plant pathogen	2-pyrrolidinone concentration (mg)									
	0.064	0.128	0.192	0.256	0.320	0.384	0.448	0.512	0.576	0.640
C. lenata	0.00	0.00	0.10	0.11	0.13	0.16	0.18	0.20	0.23	0.25
F. oxysporum	0.00	0.00	0.00	0.12	0.16	0.23	0.26	0.30	0.34	0.35



a **b** **c**

Fig. 4a Represents the control petriplate with potato dextrose agar, Fig. 4b represents the 0.32 mg concentration of 2-pyrrolidinone against *F. oxysporum* which shows a zone of inhibition of 0.16 cm and Fig. 4c represents the 0.64 mg concentration of 2-pyrrolidinone against *F. oxysporum* which shows a zone of inhibition of 0.35 cm

Inhibition curves were plotted between the zone of inhibition on the y-axis and concentration of 2-pyrrolidinone on x-axis between the bacterial and fungal plant pathogens. It was observed that the inhition activity of *P. syringae* and *F. oxysporum* is more than the other species of plant pathogens and the inhibition curves are shown in Figure 5 and 6.

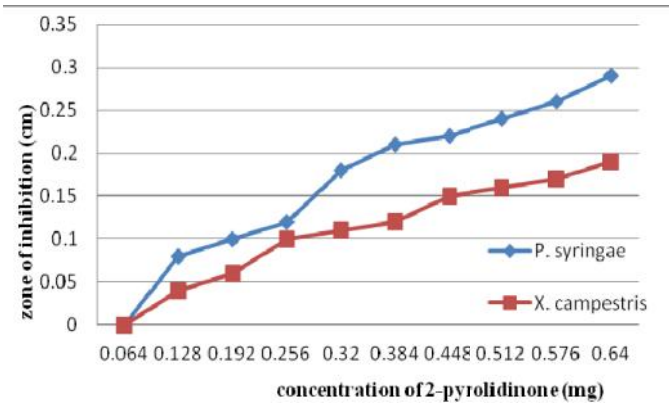


Fig. 5. Represents the inhibition curve where the blue colour represents the *P. syringae* plant pathogen and the red colour represents the *X. campestris* species

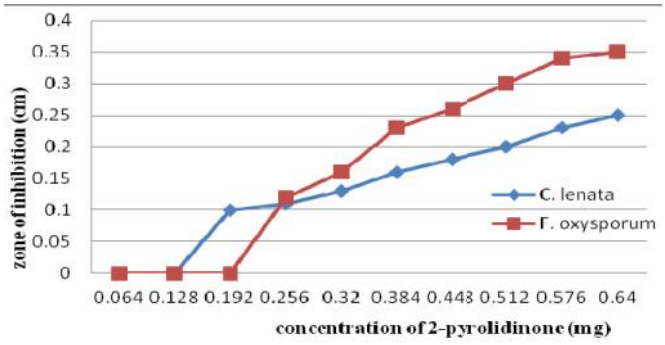


Fig. 6. Represents the inhibition curve where the blue colour represents the *C. lenata* plant pathogen and the red colour represents the *F. oxysporum* species

Conclusion

The use of pesticides may cause some undesirable effects to the ecosystem and also to human health due to the presence of pesticide residues in food. Hence several research have been promoted around the world for developing a safer and ecofriendly biopesticides. In our present study, a biocontrol agent obtained from the web of *Nephila antipodiana* was analysed by GC-MS. The effect of the alkaloid 2-pyrrolidinone has been found to inherit an anti-microbial action against bacterial and fungal plant pathogens. The organic compound 2-pyrrolidinone was observed to be more effective at higher concentrations than at lower concentrations. The inhibition curves were plotted and it was identified that amongst the two bacterial pathogens studied, *P. syringae* showed more antibacterial activity than X. campestris. *F. oxysporum* favoured anifungal action of 2-pyrrolidinone more than *C. lenata*. Similar extent of antimicrobial activity was shown by 2-pyrrolidinone which proves as an alternative to chemical pesticides. The activity of 2-pyrrolidinone can further be studied as a novel biopesticides for further studies.

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