IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

EVALUATION OF THE ANTIBACTERIAL ACTIVITIES OF COW URINE AND STREPTOMYCIN AGAINST THE FRESHLY ISOLATED STRAINS OF *XANTHOMONAS AXONOPODIS.*

Monal V. Ingawale, Sandhya Patil, Sandhya Airani, Mohan Waman

Assistant Professor, Assistant Professor, Assistant Professor, Principal
Department of Microbiology
Dr. D. Y. Patil Arts Commerce and Science College, Akurdi, Pune, India.

Abstract: Pomegranate (*Punica granatum*) belonging to the family *Punicaceae* is one of the favorite table fruit of tropical and sub-tropical regions. As a cultivated fruit crop, the Pomegranate is grown to a limited extent in selected location in many states in India. In the recent years it has been found that one of the diseases which is affecting the quality and yield of pomegranate to the large extent is oily spot disease of pomegranate. The disease is caused by *Xanthomonas axonopodis pv.punicea*. The main damage is observed on fruits, which develop black spots and usually split resulting in enormous yield losses. The pathogen is spread by infected plant material (including fruit and Cutting), mechanical means and wind –splash rain. The disease affects all above ground plant parts including flowers, leaves, twigs, stem, buds fruits and though it is more destructive. The search for the new agents or search for the agents enhancing the antimicrobial activity of the existing agents assumes the importance. In the present work , isolation of *Xanthomonas axonopodis pv .punicea*, etiological agent of oily spot disease of pomegranate was done and antimicrobial potential of streptomycin, cow urine and a combination of cow urine + streptomycin was studied. It was observed that cow urine in undilutated form (100%) could inhibit the isolate. The minimum inhibitory concentration of streptomycin for all the four isolate was found to be 9µg/ml. It was found that presence of cow urine can enhance antimicrobial potential of streptomycin to the extent of 16.6% against *Xanthomonas axonopodis pv .punicea.*

***Key words: Xanthomonas axonopodis pv. punicea* , Pomegranate , Oily Spot Disease, Cow Urine, Streptomycin.**

Introduction

Pomegranate(*Punica granatum*) belonging to the family *Punicaceae* is one of the favorite table fruit of tropical and sub-tropical regions. As a cultivated fruit crop, the Pomegranate is grown to a limited extend in selected location in many states in India. In Maharashtra, particularly in Ahmednagar, Solapur, Sangli, Pune and Wardha district with small plantation in other area. In Maharashtra state the cultivation of pomegranate is mainly confined to Solapur,Sangli and Nashik district. Pomegranate is commercially grown for its sweet acidic taste. It is a rich source of carbohydrate. Besides this, the fruit also contain protein, fiber, mineral and vitamin C. It is flavoured for its cool refreshing juice and also for its medicinal properties. The fruits are mainly used for desert purposes. The fresh fruit is of exquisite quality while its processed products, such as bottled juice, syrup and jelly are highly appreciated. Anar rub is a product locally prepared from the juice by adding sugar and heating to a thick viscous consistency. It keeps well and used like tomato sauce or ketchup.

Pomegranate is affected by many disease such as Glomerella leaf spot, fruit rot, fruit spot and canker. In the recent years it has been found that one of the diseases which is affecting the pomegranate very badly is **Oily Spot Disease of Pomegranate** (also called bacterial blight ,black spot) is caused by *Xanthomonas axonopodis pv .punicea*. The main damage is observed on fruits, which develop black spots and usually split resulting in enormous yield losses. The pathogen is spread by infected plant material (including fruit and Cutting), mechanical means and wind –splash rain. Heavy rains are thought to be a triggering factor for previous epidemic in India. No commercial pomegranate cultivar has been found to show any resistance to oily spot disease. Disease management includes use of clean planting material, phytosanitary measures and bactericidal sprays containing antibiotic or copper. However, the later may lead to rejection of exported fruit by some countries. This disease is responsible for reduction of fruit weight and export quality by means of destruction of fruit appearance. The disease affects all above ground plant parts including flowers, leaves, twigs, stem, buds fruits and though it is more destructive when fruits are

attacked. All commercially grown cultivar are susceptible to this disease. On an average the disease is reported to cause 30-50% losses. However, under favorable environmental condition 80-100% losses are also reported. The disease was found to cause following symptoms on leaves, twig, flower and fruits. Initially small (1to 2mm diameter) regular to irregular grayish black water soaked (oily spots) lesion appear on the leaves .These spots increases in size upto 3 to5mm in size in due course of time. The spots were circular, grayish black in colour oily at the center. The affected leaves then turned yellow and dropped down. Two or more spots on fruits coalesced each other led to necrosis of the rind which ultimately cracked the fruits. The infection spread from infected stem, leaves and fruits adjoining parts. Though the several chemical mixture and antibiotic are used to control the disease. Though the several chemical mixtures use to control the disease [Hingorani and Metha, (1952); Thirumalachar et al.,(1956); Rangaswami et al.,(1959);]. There are reports that the resistance to these agents is also occurring in the organism (R. Kumar, R. Shamrao Jahagirdar, Patil H. B., 2006) and therefore search for the new agents or search for the agents enhancing the antimicrobial activity of the existing agents assumes the importance. Though, antibiotics streptomycin group are routinely recommended for the management of bacterial disease, their use is not economically feasible. Extract of several plant species, weds such as (Adulsa) *Adahatoda vasica* were tried and reported effective against clinical and plant pathogens.

Also, there are reports that the resistance to these agents is also occurring in the organism and therefore search for the new agent or search for the agents enhancing the antimicrobial potential of the existing agent has become significant. In the present work, isolation of *Xanthomonas axonopodis*, etiological agent of oily spot disease of pomegranate was done and antimicrobial potential of cow urine and a combination of cow urine + streptomycin was studied.

Aims and Objective:

1) Isolation of etiological agent of diseased pomegranate sample.
2) Characterisation and identification of the isolate.
3) Evaluation of the sensitivity of the isolate to streptomycin, cow urine and combination of cow urine and streptomycin.

Material and Methods:

1) Collection of disease pomegranate sample
The disease pomegranate samples were collected from Solapur, Sangli, Satara district and also from karad local market.

2) Isolation
Isolation of organism was done by using streak plate technique and using potato dextrose Agar as growth medium and the plate were incubated at 28°C for 2-3 days .

3) Characterisation and identification
Characterisation of organism was done by studying cultural, morphological and staining properties and also by studying their physiological and biochemical characteristics as per method described by Cruikshank
Identification of isolates was done from their morphological, cultural ,staining characteristics and biochemical characteristics and with reference to Bergeys Manual of Systemic bacteriology volume I.

4) Sensitivity of the organism to the streptomycin was evaluated by agar cup diffusion method.

5) Evaluation of antibacterial activity of fresh and old cow urine against isolate was done by poison food technique using Nutrient Glucose Agar as the medium.

Results and discussion:

1) Isolation ,Characterization and identification of the organism:
From the diseased sample of promegranate that were collected from Satara, Sangli and Solapur district. Four distinct strains of *Xanthomonas axonopodis pv punicae* were isolated and they were designated as P1,P2,P3 and P4.

2) Cultural, Morphological and Biochemical Characterization of *Xanthomonas* isolate:

Table 4.1: Cultural characteristics of *Xanthomonas* isolate:

Isolate	size	shape	colour	margin	elevation	opacity	consistency
P1	2mm	circular	yellow	Entire	Raised	Smooth	Moist
P2	2mm	circular	yellow	Entire	Raised	Smooth	Moist
P3	2mm	circular	yellow	Entire	Raised	Smooth	Moist
P4	2mm	circular	yellow	Entire	Raised	Smooth	Moist

Table 4.2: Staining Properties of *Xanthomonas* isolate:

Isolate	P1	P2	P3	P4
Gram nature	Gram negative	Gram negative	Gram negative	Gram negative
Motility	Motile	Motile	Motile	Motile

Table 4.3: Result of Biochemical Properties of *Xanthomonas* isolate:

Isolate	P1	P2	P3	P4
Catalase	+	+	+	+
Starch hydrolysis	+	+	+	+
Casein hydrolysis	-	-	-	-
Gelatin hydrolysis	-	-	-	-
Oxidase	-	-	-	-
IMViC				
Indol production	-	-	-	-
Methyl red	+	+	+	+
Voges –proskauer	-	-	-	-
Citrate utilization test	-	-	-	-
Sugar Fermentation				
Glucose	+	+	+	+
Galactose	+	+	+	+
Fructose	-	-	-	-
Sucrose	+	+	+	+
Mannitol	-	-	-	-

3) Evaluation of antimicrobial activity of cow urine against *Xanthomonas* isolate

Table 4.4: Result of poison food technique against *Xanthomonas* isolate.

Urine Concentation used (v/v)	Growth of P3 isolate
control	++++
10%	+++
25%	+++
50%	++
75%	+
100%	-

Note + signs indicate extent of growth,-sign indicate no growth

4) Evaluation of antibacterial activity of cow urine in terms of that of streptomycin:

Antibacterial activities of cow urine in terms of that of streptomycin was recorded in table:

Table 4.5: Result of antibacterial activities of cow urine against *Xanthomonas* isolate.

Formulation	Diameter of zone	Equivalent to standard streptomycin concentration
Fresh Cow urine	12mm	16µg/ml
Old cow urine	14mm	20µg/ml

Antibacterial activity of fresh cow urine was shown to equivalent to 16µg/ml streptomycin concentration and antibacterial activity of old cow urine was shown to equivalent to 20µg/ml streptomycin concentration

5) Effect of cow urine on the antibacterial activity of streptomycin:
Result of effect of cow urine on the antibacterial activity of streptomycin were shown in table

Table 4.6: Result of effect of cow urine on the antibacterial activity of streptomycin against *Xanthomonas* isolate

Percentage of cow urine used(v/v) with 50 µg/ml of streptomycin	Diameter zone of inhibition(mm)
50 µg/ml of streptomycin without cow	16.6
10%	16.6
25%	16.8
50%	17.0
75%	17.6
85%	18.3

From the above table it can be seen that, as concentration of cow urine increases within sub lethal range effect of 50µg/ml of streptomycin was increasing .

Concentration of streptomycin in µg/ml	Diameter of zone of inhibition
10	11
20	14
40	17
60	18
80	20
100	22
120	23

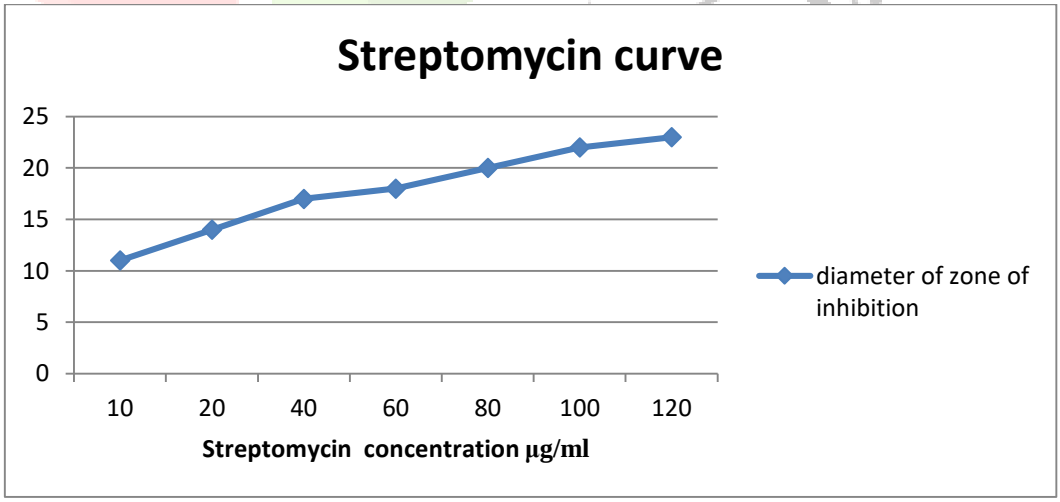

Fig no.4.1 Streptomycin curve against *Xanthomonas* isolate.

Conclusion:-
The cow urine seems to enhance the antibacterial potential of streptomycin against *Xanthomonas axonopodis pv.punicae* etiological agent of oily spot disease of pomegranate to the extent of 16.6%.

Bibliography:-

1) Bambode R.S. and Shukla V. V. (1973). Antifungal properties of certain plant extracts against some fungi. P. K. V. Res. 1, 2 (1): 1 – 8.
2) Bhardwas C. L., Prakash S. And Jamwal R. S. (1991). Differential toxicity of streptomycin to pea cultivars and its role in the management of bacterial blight, MycoI. PI. Pathol, 21:90 –91.
3) Burkholder W. H. (1948), Bacterial as plant pathogen, Ann. Rev. Microbial. 2:389 – 412.
4) Desai S. G., Patel M. K. And Desai M. V. (1967), In vitro activity of streptocycline against bacterial plant pathogen, Indian Phytopath, 20:269-300.
5) EI – Nemr, S. E., Ismail L. A. and Ragab M. (1990), Nahrugng, 34:601606.
6) EIrod R. P. And Braun A. C. (1947), Serological studies of the genus *Xanthomonas* J. Bact, 54: 349-357.
7) Hulloi S. S., Sinhg R. P and Verma J. P. (1998), Management of bacterial blight of cotton induced by Xanthomonas axonopodies pv. Malvacearum with the use of neem based formulation, Indian phytopath, 51(1):21 – 25.
8) Kanwar Z. S., and Thakur D. P. (1973), Science and culture, Some new fruit rots of pomegranate in Harayana, 39(6): 274 – 276.
9) Manjula C. P., Khan, A.N. A. and Kumar, M. R. R. (2003), Management of bacterial blight of pomegranate (Punica grantum L.) caused by *xanthomonas* axonopodies pv. Punicae. Indian phytopath, 56(30: 342.
10) Patel M. K., Srinivasan M. C. And Thirumalachar M. J. (1965), Evaluation of host specificity character in the differentiation of *Xathomonas* species. Indian phytopath, 18:78-180.
11) Rangaswami G., Rao R. R. And Lakshmanan A. R. (1959), Studies on the control of citrus canker with streptomycin phytopath, 49:224-226.
12) Sharma R. B., Sinha B. P. And Ray A. N. (1981), Post harvest fruit rot of *Punica grantum.* Two new record, Indian phytopath, 34:89 – 90.
13) Patel M. K. M. C. And Thirumalachar M. J. (1962), Two new phytopathogenic bacteria on Verbenacous host. Proc. Indian Acad. Sci. Sect. B.,56(2):88-92.
14) Srivastava M. P. (1971), Aspergillus rot of pomegranate, Indian phytopath, (XXIV)1:172.
15) Thirumachar M. J., Patel M. K., Kulkarni N. B. And Dhande G. W. (1956), Effect in vitro of some antibiotics on thirty two *xanthomonas species* occurring in India. Indian phytopath, 46:486 – 488.
16) Thrimurthy V. S. And DevadathS. (1981), Studies on the transmission and Survival of *xanthomonas* campestris pv.oryzae through insect, Indian phytopath, 34;162-163.
17) Vasudeva R. S. (1956), The agent of bacterial leaf spot of pomegranate Sci. Rep., Indian Agric. Res.Inst., New Delhi., 55 – 92.
18) Vauterin L, Hoste B, Kersters K. And Swings J. (1995), Reclassification of *xanthomonas* Int.J. Syst. Bacterial., 45:472 – 489.
19) Verma J. P. (1986), Bacterial blight of cotton, CRC press, Boca Raton, Florida, U. S. A., 278 pp.
20) Handbook of media, stains and reagents in microbiology by A. M. Deshmukh.