IJPS INTERNATIONAL JOURNAL OF PLANT SCIENCES
e ISSN-0976-593X Volume **10** | Issue 1 | January, 2015 | 19-23
**DOI: 10.15740/HAS/IJPS/10.1/19-23**
Visit us - *www.researchjournal.co.in*

**RESEARCH ARTICLE**

# Integrated management of bacterial blight disease (oily spot) of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae*

■ **C.V. AMBADKAR, A.S. DHAWAN AND V.N. SHINDE**

## SUMMARY

A study of efficacy of different antibiotics for management of bacterial blight disease of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* was conducted during the year 2010-12 at College of Agriculture, Osmanabad. *In vitro* study revealed that antibiotic streptocycline showed maximum inhibition zone of 22.21 and 31.60 per cent at 250 and 500 ppm concentrations against *X. axonopodis* pv.*punicae*, followed by tetracycline (18.26 and 27.53 %) and bacterinol (17.40 and 27.15 %) the least inhibition of bacterial growth was observed in cefaclore (13.08 and 17.53 %), respectively. Among seven botanicals neem oil showed maximum inhibition at all concentration (5, 10, 15 and 20 %) followed by garlic, neem leaf extract, tulasi leaf extract, ginger extract, guava leaf extract and aloe vera, respectively. The bacterial antagonistic *viz.*, *Pseudomonas fluorescens* and *Bacillus substilis* were found effective in inhibiting the test pathogen at 15.43 and 12.71 per cent, respectively. Based on the efficacy of these different antibiotics, bioagents and plant extracts, the best one were applied in integrated management schedule for mitigating bacterial blight of pomegranate. The schedule was applied at five different locations in Marathwada region of Maharashtra. At the time of adoption of orchards, the per cent disease severity observed in the orchard at Kelewadi, Wagholi, Sakanewadi, Hol and Killari were 16.44, 16.56, 17.85, 11.50 and 26.32 which was reduced to 3.5, 8.23, 8.14 and 9.53 and 9.04, respectively at harvest.

**Key Words :** *Xanthomonas axonopodis* pv. *punicae*, Antibiotics, Integrated management, Bacterial blight

**How to cite this article :** Ambadkar, C.V., Dhawan, A.S. and Shinde, V.N. (2015). Integrated management of bacterial blight disease (oily spot) of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae*. *Internat. J. Plant Sci.*, **10** (1): 19-23.

**Article chronicle : Received :** 20.05.2014; **Revised :** 07.11.2014; **Accepted :** 21.11.2014

Pomegranate is gaining lot of attention of the world over, because of its high economic and nutritional values. Maharashtra is the leading state in area and production of pomegranate. However, in the recent past pomegranate cultivation in Maharashtra has been threatened due to incidence of bacterial blight (oily spot) disease (*Xanthomonas axonopodis* pv. *punicae*). The disease has been resulted in enormous losses to pomegranate orchards, as it renders fruits unfit for consumption and market. The disease has spread like wildfire in the pomegranate plantation in the Maharashtra, particularly in the districts of Solapur, Sangali, Satara, Osmanabad, Latur and Beed. Hence, it is the demand of the time to search for suitable and effective control measures against the disease. Therefore, the present investigation was planned to test the efficacy of different antibiotics, plant extracts and bioagents in management of bacterial blight disease of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae.* The antibiotics, plant extracts and bioagents which were found best in these treatments were applied in integrated management schedule. The schedule of sixteen spraying was followed in

**MEMBERS OF THE RESEARCH FORUM**

**Author to be contacted :**
**C.V. AMBADKAR,** Department of Plant Pathology, College of Agriculture, OSMANABAD (M.S.) INDIA

**Address of the Co-authors:**
**A.S. DHAWAN,** Directorate of Extension Education, Vasantrao Naik Marathwada Krishi Vidyapeeth, PARBHANI (M.S.) INDIA

**V.N. SHINDE,** Department of Horticulture, College of Agriculture, OSMANABAD (M.S.) INDIA

five different pomegranate orchards located in beed, Latur and Osmanabad districts of Maharashtra state.

## MATERIAL AND METHODS

The present study was carried out at during the year 2010-12 at College of Agriculture, Osmanabad (M.S.) India.

### *In vitro* evaluation of antibiotics :

Total seven antibiotics *viz.*, streptomycine, streptocycline, rifampcin, bacterinol (2 - Bromo, 2- Nitro propane-1, 3-Diol), tetracycline, chloramphenicol, cephaclore were tested against *Xanthomonas axonopodis* pv. *punicae* by using Completely Randomized technique with three replications. Each chemical at concentration 250 and 500 was evaluated *in vitro* applying inhibition zone technique (paper disc method) and using Nutrient agar (N.A.) as basal culture medium.

Observations on radial growth of test pathogen and per cent inhibition over control was calculated by the formula of Vincent (1947) and Ashoka *et al.* (1982) :

$$I = \frac{C - T}{C} \times 100$$

where,

I = Per cent inhibition

C = Growth of test pathogen in control plate

T = Growth of test pathogen in treatment plate.

### *In vitro* evaluation of botanicals and biocontrol agents :

Plant leaf extract of seven botanicals *viz.*, neem leaf extract, neem oil, guava leaf extract, tulasi, ginger extract, garlic extract, aloe vera extract and two biocontrol agents *viz.*, *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated against *X. axonopodis* pv. *punicae* in Completely Randomized technique with three replications. Before preparation of leaf extract, leaves of each plant species were dipped in the 0.1per cent mercuric chloride ($HgCl_2$). Leaf extracts were prepared by grinding 100g washed leaves of each plant species in 100ml distilled water with mixture-cum grinder and filtered through Whatman number 1 filter paper. These extract were refiltered through Seitz filter. The final clear filtrate obtained was treated as 100 per cent concentration of standard leaf extract. The desired quantity required for preparation of 5, 10, 15 and 20 per cent concentration was taken from this 100 per cent standard leaf extract. These leaf extracts were then evaluated *in vitro* against *Xanthomonas axonopodis* pv. *punicae* by applying inhibition zone technique.

Bacterial antagonists *viz.*, *Pseudomonas fluorescens* and *bacillus subtilis* were evaluated *in vitro* against *Xanthomonas axonopodis* pv. *punicae* applying inhibition zone technique. The pure culture of *Pseudomonas fluorescens* and *bacillus subtilis* were obtained from Department of Plant Pathology, Marathwada Agricultural University, Parbhani multiplied and maintained in nutrient agar media in laboratory for further studies. Seven day-old-culture of bioagents antagonists grown on NA were used for the study. Disc (5 mm dia.) of NA along with culture of the bioagents were cut out with sterilized cork borer. Then culture disc one of each of the bioagent was placed at centre on solidified plates containing nutrient agar

**Table A : Spray schedule followed**

| No. of spray | Time of spray | Chemicals used for spray |
|---|---|---|
| 1st | Immediately after pruning | Bordeaux mixture (1%) |
| 2nd | Seven days after Ist spray | *Pseudomonas fluorescence* talc based formulation (2g/l) |
| 3rd | Eight days after II nd spray (New flush stage) | Copper oxychloride 50WP (2.5g/l) + Bronopol (o.5g/l). Thiomethoxam 25WG (o.3g/l) for sucking pest. |
| 4th | 15 days after 3rd Spray (Flower initiation stage) | Streptocycline (0.5g/l) + Carbendazim 50WP (1g/l) + Acetamiprid 20SP (0.3g/l). Spray of micronutrient mixture |
| 5th | 15 days after 4th spray | Captan 50WP (2.5g/l) + Bronopol (0.5g/l) + Imidacloprid 17.8SL (0.3ml/l) |
| 6th | 15 days after 5th spray (Fruit initiation stage) | Streptocycline (0.5g/l)+ Thiophanate methyl 70WP (1g/l) + Cypermethrin 25EC (1ml/l)+ Micronutrient mixture (1g/l)+ NSKE (50g/l) |
| 7th | Seven days after 6th spray | Pseudomonas fluorescence talc based formulation (2g/l) |
| 8th | Seven days after 7th spray | Bordeaux mixture (0.5%) |
| 9th | 15 days after 8th spray (50% Fruit set) | Streptocycline (0.5g/l) + Carbendazim 50WP (1g/l) + Chloropyriphos 20EC (2ml/l)+ NSKE (50g/l) |
| 10th | 15 days after 9th spray (100% Fruit set) | Bordeaux mixture (0.5%) |
| 11th | 15 days after 10th spray | Captan 50WP (2.5g/l) + Bronopol (0.5g/l) + Methomyl 40SP (1g/l) |
| 12th | 15 days after 11th spray | Streptocycline (0.5g/l) + Thiophanate methyl 70WP (1g/l)+ Acetamiprid 20SP (0.3g/l) |
| 13th | 15 days after 12th spray | Bordeaux mixture (0.5%) |
| 14th | 15 days after 13th spray | Streptocycline (0.5g/l) + Copper hydroxide 77WP(2g/l)+ NSKE (50g/l) |
| 15th | 15 days after 14th spray | Pseudomonas fluorescence talc based formulation (2g/l) |
| 16th | 15 days after 15th spray | Potassium dihydrogen phosphate (5g/l) or Potassium nitrate (10g/l) or 0:0:50 (10g/l) |

with bacterial suspension under aseptic conditions and plates were incubated at 27± 2$^0$C. Plates inoculated with the culture disc of test pathogen were maintained as untreated control. Observation of test pathogen and bioagent were recorded at interval of 72 hours. The per cent inhibition was evaluated by applying the formula Ashoka *et al*. (1982) :

$$I = \frac{C - T}{C} \times 100$$

where,

I = Per cent inhibition
C = Growth of test pathogen in control plate
T = Growth of test pathogen in treatment plate.

**Integrated management of bacterial blight of pomegranate *in vivo* :**

The antibiotics, bioagents and plant extracts along with micronutrients and insecticides were used in integrated management schedule. This schedule was prepared in Network Project on Mitigating Bacterial Blight disease of Pomegranate in Maharashtra. For effective application of this schedule, five different orchards at different locations were adopted in the month of Oct. 2011 in beed, Latur and Osmanabad districts. The per cent disease severity before adoption of these orchards was measured which was upto 50 per cent. The pruning operation was undertaken in the second week of September, 2011 (Hol), November 2011 (Wagholi, Sakanewadi and Killari) and first week of January, 2012 (Kelewadi). In these demonstration plots the schedule of management of bacterial blight of pomegranate was strictly followed. The per cent disease severity before adoption of these orchards and after following the spray schedule was measured.

## RESULTS AND DISCUSSION

The result presented in Table 1 revealed that antibiotic streptocycline at 250 ppm concentration was found most effective for controlling *X. axonopodis* pv. *punicae* by forming

**Table 1: Inhibitory effect of antibiotics on growth of *X. axonopodis* pv. *punicae***

| Treatments | Mean bacterial growth (mm)*at conc. | | % inhibition of bacterial growth | |
|---|---|---|---|---|
| | 250ppm | 500ppm | 250ppm | 500ppm |
| Streptomycin | 76.89 | 69.11 | 14.56 (8.37) | 23.21 (13.42) |
| Streptocycline | 70.00 | 61.55 | 22.21 (11.28) | 31.60 (18.42) |
| Rifampcin | 77.33 | 73.56 | 14.07 (8.09) | 18.26 (10.52) |
| Bacterinol | 74.34 | 65.55 | 17.40 (11.00) | 27.15 (15.76) |
| Tetracycline | 73.56 | 65.52 | 18.26 (11.05) | 27.53 (15.98) |
| Chlorampehnicol | 77.22 | 71.89 | 14.19 (8.16) | 20.11 (11.60) |
| Cefachlore | 78.22 | 74.22 | 13.08 (7.51) | 17.53 (10.09) |
| Control | 90.00 | 90.00 | 0.00 | 0.00 |
| S.E. ± | 0.44 | 0.50 | 0.49 | 0.63 |
| C.D.(P=0.05) | 1.32 | 1.51 | 1.47 | 1.91 |

* Mean of three replications Figures in parenthesis are arcsin values

**Table 2: Inhibitory effect of botanicals(5 and 10 %)and bioagents on growth of *X. axonopodis* pv. *punicae***

| Treatments | Mean bacterial growth (mm)*at conc. | | | | % inhibition of bacterial growth | | | |
|---|---|---|---|---|---|---|---|---|
| | 5 % | 10 % | 15 % | 20 % | 5 % | 10 % | 15 % | 20 % |
| Neem leaf extract | 83.44 | 79.22 | 78.22 | 74.89 | 7.28 (4.17) | 11.97(6.87) | 13.08(7.51) | 16.78(9.66) |
| Neem oil | 82.22 | 77.78 | 75.99 | 71.92 | 8.64(4.96) | 13.58(7.80) | 15.57(8.95) | 20.09(11.59) |
| Guava leaf extract | 85.33 | 81.00 | 80.56 | 79.39 | 5.18(2.97) | 9.99(5.74) | 10.49(6.02) | 11.78(6.76) |
| Tulasi leaf extract | 83.61 | 79.55 | 78.65 | 76.04 | 7.09(4.06) | 11.60(6.66) | 12.60(7.23) | 15.51(8.92) |
| Ginger extract | 83.78 | 80.11 | 78.91 | 77.11 | 6.91(3.96) | 10.98(6.31) | 12.32(7.07) | 14.31(8.23) |
| Garlic extract | 83.22 | 78.99 | 77.50 | 73.99 | 7.53(4.32) | 12.22(7.02) | 13.88(7.98) | 17.79(10.24) |
| Aloe vera extract | 86.11 | 83.93 | 82.74 | 80.82 | 4.32(2.47) | 6.79(3.89) | 8.05(4.62) | 10.19(5.85) |
| *Pseudomonas fluoroscens* | - | 76.11 | -- | -- | - | 15.43(8.88) | | |
| *Bacillus subtilis* | - | 78.55 | -- | -- | - | 12.71(7.30) | | |
| Control | 90.00 | 90.00 | 90.00 | 90.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| S.E. ± | 0.63 | 0.59 | 0.35 | 0.44 | 0.70 | 0.65 | 0.39 | 0.49 |
| C.D.(P=0.05) | 1.90 | 1.74 | 1.05 | 1.34 | 2.11 | 1.91 | 1.17 | 1.48 |

* Mean of three replications Figures in parenthesis are arcsin values

22.21 per cent inhibition zone. Tetracycline was found second best effective antibiotics which showed 18.26 per cent inhibition followed by bacterinol 17.40 per cent, streptomycine 14.56 per cent, chloramphenicol 14.19 per cent, rifampcin 14.07 per cent and cefaclore 13.08 per cent inhibition zone. Similarly, at 500 ppm concentration streptocycline was found most effective for controlling *X. axonopodis* pv. *punicae* by forming 31.60 per cent inhibition zone. Tetracycline was found second best effective antibiotics which showed 27.53 per cent inhibition followed by bacterinol 27.15 per cent, streptomycine 23.21 per cent, chloramphenicol 20.11per cent, rifampcin 18.26 per cent and cefaclore 17.53 per cent inhibition zone. Similar results were reported by Suryawanshi *et al*. (2009).

**Botanicals and bio agents :**

*In vitro* evaluation of seven botanicals *viz.*, neem leaf extract, neem oil, guava leaf extract, tulsi, ginger extract, garlic extract, aloe vera extract @ 5,10,15 and 20 per cent and two bio agents *viz*., *Pseudomonas fluorescens* and *Bacillus subtilis* (culture disc 5 mm each) were carried out for control of *X. axonopodis* pv. *punicae* by using inhibition zone technique.

The result presented in Table 2 revealed that neem oil at 5,10, 15 and 20 per cent was found most effective for controlling *X. axonopodis* pv. *punicae* by forming 8.64, 13.58, 15.57 and 20.09 per cent inhibition, respectively. Garlic extract was found second best effective botanicals which showed 7.53, 12.22, 13.88 and 17.89 per cent inhibition at 5,10.15 and 20 per cent, respectively. Among plant extracts minimium inhibition at 5,10,15 and 20 per cent was observed in the treatment of aloe vera extract *i.e*. 4.32, 6.79, 8.05 and 10.19 per cent inhibition, respectively.

The result presented in Table 2 revealed that bio agent *Pseudomonas fluoroscens* was found most effective for controlling *X. axonopodis* pv. *punicae* by forming 15.43 per cent inhibition followed by *Bacillus subtilis* with 12.71 per cent inhibition.

The results obtained in present investigation are in full agreement with those reported in the past. Desai *et al*. (1967) evaluated *in vitro* activity of streptocycline against 17 *Xanthomonas* species and two *Pseudomonas* species with different concentrations (25, 50, 100, 250 ppm). The results indicated that streptocycline in all concentrations tried (25, 50, 100, 250 ppm) inhibited the growth of all the bacterial plant pathogens belonging to *Xanthomonas* and *Pseudomonas* genera comprising 19 species. Among the *Xanthomonas, X. lawsoniae*, *X. ionidi*, *X. nakatae-corchori* and *X. patelii* are more sensitive when compared to other 13 species. Similar results have been reported by Premalatha Dath and Devadath (1969); Gandhi and Parashar (1978); Nath *et al.* (1979); Sharma *et al.* (1979); Ravikumar *et al.* (2003); Manjula *et al.* (2003) ; Atar (2011); Giri *et al.* (2008); Patil *et al*. (2006); Raut *et al.* (2010) and Hulloli *et al.* (1998).

**Integrated management of bacterial blight of pomegranate *in vivo* :**

At the time of adoption of orchards, the per cent disease severity observed in the orchard at Kelewadi, Wagholi,

**Table 3: Comparison of per cent disease severity on tree before adoption of the orchards and at the time of harvest**

| Sr. No. | Demonstration sites | Area in ha | Adopted demonstration plots | | | Non adopted plots | |
|---|---|---|---|---|---|---|---|
| | | | Per cent disease severity on tree before adoption | Per cent disease severity on tree after adoption (At harvest) | Yield/ha (tones) | Per cent disease severity on tree (At harvest) | Yield/ha (tones) |
| 1. | At. Kelewadi Po. Pardi Tq. Washi Dist.Osmanabad | 1.0 | 16.44 | 3.5 | 5.10 | 16.87 | 3.80 |
| 2. | At.Po. Wagholi Tq. Osmanabad Dist. Osmanabad | 1.0 | 16.56 | 8.23 | 3.58 | 21.59 | 2.10 |
| 3. | At. Po. Sakanewadi Tq. Osmanabad Dist. Osmanabad | 1.0 | 17.85 | 8.14 | 4.25 | 21.59 | 2.10 |
| 4. | At. Po. Hol Tq. Kej Dist. Beed | 1.0 | 11.50 | 9.53 | 4.20 | 21.23 | 3.60 |
| 5. | At. Wanewadi Po. Killari Tq. Ausa Dist. Latur | 1.0 | 26.32 | 9.04 | 6.80 | 20.99 | 4.00 |

Sakanewadi, Hol and Killari were 16.44, 16.56, 17.85, 11.50 and 26.32 which was reduced to 3.5, 8.23, 8.14 and 9.53 and 9.04, respectively at harvest by application of integrated management schedule (Table 3). The disease severity in adjoining plots where the integtrated spray schedule was not followed were 16.87, 21.59, 21.59, 21.23 and 20.99 at the time of harvest. The harvesting of fruits in above orchards was done in the month of April, July, August and September, 2012. The maximum marketable fruit yield (6.80 tones/ha) was obtained from the demonstration plot adopted at Wanewadi (Killari) followed by Kelewadi (5.10 tones/ha), Sakanwadi (4.25 tones/ha), Hol (4.20 tones/ha) and Wagholi (3.58 tones/ha) with minimum loss by bacterial blight disease of pomegranate, whereas in non - adopted orchards the fruit yield was in the range of 2.10 to 4.00 tones only (Fig. 1). From the above results it is clear that the integrated spray schedule was found very effective in management of bacterial blight of pomegranate.

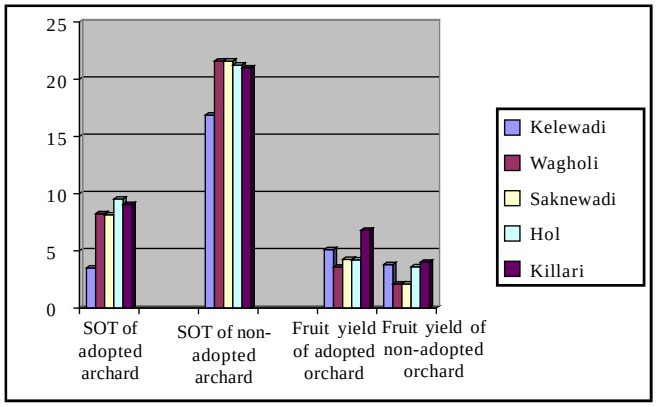


**Fig. 1: Comparison of per cent disease severity on tree before adoption of the orchards and at the time of harvest**

## REFERENCES


Atar, M.A.(2011). Efficacy of different antibiotics and botanicals for controlling bacterial blight (*Xanthomonas axonopodis* pv. *punicae*) of pomegranate.M.Sc. (Ag.) Thesis, Marathwada Agricultural University, Parbhani, M.S. (INDIA).

Basavaraj, Khan, A.N.A., Kiran, Kumar, K.C. and Shreenivasa, K.R. (2007). Biological control of bacterial blight of pomegranate. National Symposium on Potentials of Biological Control Agents in Agriculture: Prospects and Perspectives. held at College of Agriculture, Nagpur on Oct.; 27th and 28th. pp.51.

Desai, S.G., Patel, M.K. and Desai, M.V. (1967). *In vitro* activity of streptocycline against bacterial plant pathogens. *Indian Phytopath.*, **20**: 296-300.

Gandhi, S.K. and Prashar, R.D. (1978). Evaluation of some fungicides and antibiotics against *Xanthomonas campestris*, causing bacterial rot. *Indian Phytopath.*, **31**: 210-213.

Giri, G.K., Gade, R.N., Gulhane, A.R. and Das, Supriya (2008). Efficacy of bioagents and botanicals and chemicals against citrus canker (*X. axonopodis* pv. *citri.*) *J.Pl.Dis.Sci.*, **3** (2): 249 - 250.

Hulloli, S.S., Singh, R.P. and Verma, J.P. (1998). Management of bacterial blight of cotton induced by *Xanthomonas axonopodis* pv *malvacearum* with use of neem based formulations. *Indian Phytopath.*,**51**(1):21-25.

Jagunatharaddi, J.N., Khan A.N.A., Kumar, K.C. and Basha,C.R. (2007). *In vitro* and *in vivo* evaluation of biocontrol agent against bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae*. National Symposium on Potentials of Biocontrol Agents in Agriculture: Prospects and Perspctives, held at College of Agriculture, Nagpur on Oct-27th and 28th pp.51.

Manjula, C.P., Khan, A.N.A. and Ravikumar, M.R. (2003). Management of bacterial blight of pomegranate (*Punica granatum* L.) caused by *Xanthomonas axonopodis* pv. *punicae*. *Indian Phytopath.*, **56** (3): 341.

Nath, J., Gurha, S.N. and Taneja, N.K. (1979). Sensitivity of *Xanthomonas malvacearum* the incitant of bacterial blight of cotton to chemicals. *Indian J. Mycol. & Pl. Pathol.*, **11**(2): 255-257.

Patil, Minakshi, Deshmukh, V.V., Sawargaonkar, G.L., Patil, Manjusha and Borgaonkar, S.B. (2006). Efficacy of different chemicals, antagonists and botanicals against bacterial blight of cotton. *J. Pl. Dis. Sci*., **1**(2) : 205-206.

Premalatha, Dath A. and Devadath, S. (1969). *In vitro* effect of antibiotics against *Xanthomonas translucens* f. sp. *oryzae*. *Indian Phytopath.*, **22**: 386-388.

Raut, S.A., Ingole O.V., Raut, B.T. and Bhoye, B.B. (2010). Evaluation of bioagents, botanicals and chemicals against bacterial blight of cotton. *J. Pl. Dis. Sci*., **1**: 83-85.

Ravikumar, M.R., Jahagirdar, S. and Khan, A.N.A. (2003). Management of bacterial leaf spot of grape through chemicals and antibiotics in Northern Karnataka. *Indian Phytopath*., **56** (3): 341.

Sharma, R.R., Thind, B.S. and Singh, Nirmaljit (1979). *In vitro* and *in vivo* evaluation of chemicals against *Xanthomonas vesicatoria*, the causal agent of bacterial leaf spot of chillies. *Indian J. Mycol.& Pl. Pathol.*, **11**(2):178-182.

Suryawanshi, A.P., Mogle, T.R., Somwanshi, S.D., Magar, S.J. and Wadulkar, B.D.(2009). Efficacy of antibiotics and fungicides against oily spot disease of pomegranate. National Conference on Frontiers in Plant Diseases, Diagnosis and Management, held at Maharashtra Mahavidyalay, Nilanga. on Jan. 3rd and 4th 28 pp.

Vincent, J.M. (1947). The use of antibiotics in the control of diseases of vegetables. *Grandinaratvo*, **10**(7):18-21.