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Evaluation of Roko in Combination with Agrochemicals against Fusarium Oxysporum Schlecht. Under in Vitro Conditions

A. M. Jamadar[1] and S. K. Khade[2]
[1]Assistant Professor, Department of Botany
[2]Professor, Department of Botany
[1]Willingdon College, Sangli, Maharashtra, India.
[2]D. K. A. S. C. College, Ichalkaranji, Kolhapur, Maharashtra, India.
[1]jafsana@gmail.com

Abstract: *Root rot is an important disease of Soybean mainly caused by Fusarium oxysporum Schlecht. which leads to significant yield loss. The present study was conducted to evaluate the effectiveness of agrochemicals in managing root rot disease of soybean. The minimum inhibitory concentration (MIC) of Roko was tested against different isolates of Fusarium oxysporum Schlecht. using the agar plate method. The MIC values ranged from 8 µg/ml to 290 µg/ml Roko with different agrochemicals such as Fungicides (Bordeaux mixture, Dhanuka, Aliette, Zeb M-45), Insecticides (Lannate, Chloropyrifos, Cannon, Malathion) and Herbicides (Goal, Weedmar, Aandhi71%, 2,4-D) was also studied. The results showed that these combinations effectively inhibited the in vitro growth of the pathogen.*

Keywords: Soybean, Fusarium oxysporum, MIC, Agrochemicals.

I. INTRODUCTION

Soybean [*Glycine max* (L.) Merrill.] is the most important legume crop in the world. It is also called 'Golden bean', 'Miracle bean' and 'Crop of planet.' Soybean is capable of fixing and utilizing atmospheric nitrogen through symbiotic relationship with *Rhizobium* bacterium, so the crop improves soil fertility and economizes crop production not only for themselves but also for the next crop grown in rotation especially, cereal crops [1].

Soybean has great nutritional value. It is easily digestible and is considered as one of the richest and cheapest source of proteins, hence called as 'Poor man's meat'. It is one of the world's most important source of oil and protein. Soybean contains 30% oil of dry seed weight and it is an important source of protein, which reaches 50% of dry seed weight along with calcium, iron, zinc, carotene, thiamine and ascorbic acid [2, 3, 4].It has great medicinal importance as soya based food helps to control diabetics and melts all kinds of stones in the urinary bladder. Seeds were primarily used as pulses by the local population and the green and dried vegetative part were used as foliage for cattle.

Soybean serve as one of the valuable crop in India with innumerable possibilities of not only improving agriculture but also supporting industries, its cultivation has increased tremendously. In India, it is grown on an area of 11.25 million hectare with a production of 14.22 million metric tonnes and productivity of 1268 kg/ha in the year 2017 [5]. Amongst the soybean producing nation, India occupies dismal fifth position in production though we are third after USA and China in area.

Among the different states in India, Maharashtra is having total area of 39.24 lakh hectare and production of 51.37 lakh metric tonnes with productivity of 1309 kg/ha. [2,4].

Soybean is known to suffer from many fungal diseases such as Root rot, Leaf spot, Charcoal rot, Damping off, Late blight, Wilt, Rusts and Smuts having a significant impact on yield and quality. Hence managing them becomes the first part of crop production [6].

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More than 68 pathogens has been found so far from different soybean growing countries [7]. Major fungal diseases of soybean are root rot (*Fusarium oxysporum* Schlecht.), leaf spot (*Alternaria alternata*), Charcoal rot (*Macrophomina phaseolina*), Damping-off (*Rhizoctonia solani*) but among these root rot of soybean is very serious disease in India which cause 80 to 90% yield loss [7]. Root rot of soybean caused by *Fusarium oxysporum* Schlecht.is one of the most serious disease which shows both the symptoms of wilt and root rot [8].

The main symptoms are spreading of roots vertically and shows dark brownish to blackish rotten patches on the internal tissues, diseased plant are get stunted, yellowing and drying of leaves and browning of vascular bundle. Drooping of petioles and rachis, yellowing and drying of leaves from base to upward, browning of vascular bundles, improper branching, withering of plants and finally death of plants [2,4]. Mycelium enters in to xylem vessels and acquire whole vascular system of host results in to blockage of water and mineral transport results in to wilting and yellowing of plant. It was observed that in the absence of host *Fusarium oxysporum* Schlecht. can survive in the soil up to five years [4]. The disease has assumed great importance in Maharashtra state during the past few years due to severe yield loss.

Fusarium oxysporum Schlecht. causing Root rot disease to soybean is severe in all pathogens in soybean. Roko, a systemic fungicide is recommended for the management of soybean diseases. Hence this fungicide was undertaken for detailed study.During present investigation Roko with other fungicides and also with insecticides and herbicide was used to control the growth of pathogen.

II. MATERIAL AND METHODS

Surveys were conducted in Soybean growing areas of five districts of Maharashtra state i.e. Sangli, Kolhapur, Satara, Pune and Solapur. The pathogen*Fusarium oxysporum* Schlecht.from the collected diseased samples isolated on Czapek Dox Agar (CDA) medium following the scientific method [9]. In all fifteen isolates of *Fusarium oxysporum* Schlecht. were obtained and used for the experiment.

In vitro sensitivity of all fifteen isolates of *Fusarium oxysporum* Schlecht. to Roko was estimated by Food Poisoning Technique [10]. The 8 mm discs of fungal culture were taken on CDA plates containing different concentrations of Roko. The plates without agrochemicals were treated as Control. Now the plates were incubated at 28±2 °C. Radial growth of fungal colonies were measured in mm till the fungal colonies fully covered the petriplates. Isolate Fo-5 (Kagal, Kolhapur) was sensitive among fifteen isolates while isolate Fo-15 (Barshi, Solapur) was the most resistant and it was selected for the study.

Roko resistant isolate Fo-15 was inoculated on the plates of agar having 25, 50, 75 and 100 µg/ml concentrations of different fungicides (Bordeaux mixture, Dhanuka, Aliette, Zeb M-45), insecticides (Lannatte, Chloropyrifos, Cannon, Malathion), herbicides (Goal, Weedmar, Andhi 71 %, 2,4-D) along with Roko (290 µg/ml). These plates were incubated at 28±2 °C. Radial growth of fungal colony was measured in mm at different intervals. Plates without any agrochemicals were treated as control.

III. RESULTS AND DISCUSSION

Table 1 shows MIC of Roko against fifteen isolates of *Fusarium oxysporum* Schlecht. MIC on agar plates ranged from 8 to 290 µg/ml. Isolate Fo-5 was sensitive and Fo-15 was most resistant nd tolerated to 290 µg/ml of Roko. Remaining isolates were moderately resistant.

Table 1 - MIC (Minimum Inhibitory Concentration) of Roko against *Fusarium oxysporum* Schlecht.*isolates* causing rootrot of soybean.

Sr.No.	Isolate	Locality	*In vitro* (µg/ml)
1.	Fo-1	Vita (Sangli)	60
2.	Fo-2	Palus (Sangli)	130
3.	Fo-3	Miraj (Sangli)	180
4.	Fo-4	Hatkanangale (Kolhapur)	40

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5.	Fo-5	Kagal (Kolhapur)	8
6.	Fo-6	Shirol (Kolhapur)	190
7.	Fo-7	Phalatan (Sartara)	20
8.	Fo-8	Patan (Sartara)	240
9.	Fo-9	Karad (Sartara)	50
10.	Fo-10	Ambegaon (Pune)	150
11.	Fo-11	Talegaon (Pune)	120
12.	Fo-12	Alandi (Pune)	80
13.	Fo-13	Akkalkot (Solapur)	110
14.	Fo-14	Pandharpur (Solapur)	210
15.	Fo-15	Barshi (Solapur)	290

Table 2 shows synergestic effect of fungisides on the development of Roko resistance in *Fusarium oxysporum* Schelcht.(*in vitro*). The fungicides like Bordeaux mixture of Shows slight growth of *Fusarium oxysporum* Schelcht. at 25µg/ml concentration. While Dhanuka, Aliette and Zeb M-45 were used in mixture with Roko (290 µg/ml) at different concentrations (10, 25, 50, 75 and 100 µg/ml) completely inhibited the growth of resistant isolate.

Table 2 - Synergistic effect of Fungicides on the development of Roko resistance in *Fusarium oxysporum* Schlecht.(*In vitro*)

Sr. No.	Fungicide with Roko 290 µg/ml	Concentrations µg/ml	Radial mycelial growth in mm
1.	Bordeaux mixture	25	05.33
		50	00.00
		75	00.00
		100	00.00
2.	Dhanuka	25	00.00
		50	00.00
		75	00.00
		100	00.00
3.	Aliette	25	00.00
		50	00.00
		75	00.00
		100	00.00
4.	Zeb M-45	25	00.00
		50	00.00
		75	00.00
		100	00.00
5.	Control (Roko alone 290 µg/ml)	-----	10.33

Table 3 shows synergestic effect of insecticides on the development of Roko resistance in *Fusarium oxysporum* Schelcht.(*in vitro*). The insecticides Lannate shows slight growth of *Fusarium oxysporum* Schelcht. at 25 µg/ml concentration. While Chloropyrifos, Cannon and Malathion were used in mixture with Roko at different concentrations (10, 25, 50, 75 and 100 µg/ml) totally supressed the growth of *Fusarium oxysporum* Schelcht..

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Table 3 - Synergistic effect of Insecticides on the development of Roko resistance in *Fusarium oxysporum* Schlecht. (*In vitro*)

Sr. No.	Insecticide with Roko 290 µg/ml	Concentrations µg/ml	Radial mycelial growth in mm
1.	Lannate	25	08.33
		50	00.00
		75	00.00
		100	00.00
2.	Chloropyrifos	25	00.00
		50	00.00
		75	00.00
		100	00.00
3.	Cannon	25	00.00
		50	00.00
		75	00.00
		100	00.00
4.	Malathion	25	00.00
		50	00.00
		75	00.00
		100	00.00
5.	Control (Roko alone 290 µg/ml)	-----	09.33

Table 4 shows that synergistic effect of the herbicides (Goal, Weedmar, Aandhi 71% and 2,4-D) were used in mixture with Roko at different concentrations (10, 25, 50, 75 and 100 µg/ml). It was recorded that all herbicides are very effective against *Fusarium oxysporum* Schlecht.

Table 4 - Synergistic effect of Herbicides on the development of Roko resistance in *Fusarium oxysporum* Schlecht. (*In vitro*)

Sr. No.	Herbicide with Roko 290 µg/ml	Concentrations µg/ml	Radial mycelial growth in mm
1.	Goal	25	00.00
		50	00.00
		75	00.00
		100	00.00
2.	Weedmar	25	00.00
		50	00.00
		75	00.00
		100	00.00
3.	Aandhi 71%	25	00.00
		50	00.00
		75	00.00
		100	00.00
4.	2,4-D	25	00.00
		50	00.00
		75	00.00

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		100	00.00
5.	**Control (Roko alone 290 µg/ml)**	-----	09.33

Ridomil with fungicides (Roko, Kavach, Carbendazim and Benomyl), insecticides (Phorate, Sunself and Prime), herbicides (Matine, Aandhi 71%, 2, 4-D and Atrazine) reduced the development of Ridomil resistance in *Rhizopusartocapi* causing fruit rot of Jackfruit [11]. It has been showed that the alternately used fungicide have different mode of action and there might be less chance to mutate or to adopt resistance in the pathogen due to use of different specific site inhibiting fungicide [12]. Benomyl in combination with Captan, Imazalil and Chlorothalonil reduced resistance in *Venturia inaequalis* causing apple scab [13].

Gleosporium amphelophagum causing anthracnose of grapes was managed by alternate use of Roko with Bordeaux mixture or Indofil M-45 [14]. It was found that the growth of*Macrophomina phaseolina* causing charcoal rot of potato was completely inhibited by the synergistic effect of agrochemicals like Mancozeb and Zineb with carbendazim [15].

Many other workers also reported similar results in case of different pathogens [16, 17, 18, 19, 20, 21 22].

IV. CONCLUSION

The synergistic effect of Roko with different agrochemicals such as Fungicides (Bordeaux mixture, Dhanuka, Aliette, Zeb M-45), Insecticides (Lannate, Chloropyrifos, Cannon, Malathion) and Herbicides (Goal, Weedmar, Aandhi71%, 2,4-D) effectively inhibited the *in vitro* growth of *Fusarium oxysporum* Schelcht. causing root rot of Soybean.

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