IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

EFFECT OF CARBENDAZIM AND OTHER FUNGICIDES ON THE DEVELOPMENT OF RESISTANCE DURING PASSAGE IN ALTERNARIA ALTERNATA (FR.) KEISSL. CAUSING ALTERNARIA BLIGHT OF CHICKPEA

[1]S. N. Inamdar and [2]M. B. Waghmare

[1]Associate Professor, [2]Professor and Head

[1]Department of Botany, Miraj Mahavidyalaya, Miraj - 416410. (M. S.)

[2]Department of Botany, The New College, Kolhapur- 416012. (M. S.)

***Abstract*:** Chickpea (*Cicer arietinum* L.) is an important pulse food crop in India. It get infected by many fungi where *Alternaria alternata* (Fr.) Keissl. was found to be dominant among the diseased samples collected during investigation. From these samples of *Alternaria alternata* (Fr.) Keissl., wild sensitive (Aa-1) and highly resistant (Aa-11) isolates were identified using Carbendazim and used for passage study. It was seen that growing of *Alternaria alternata* (Fr.) Keissl. on the medium containing Carbendazim for eight successive passages continuously, increased the resistance constantly till 8th passage. When it was cultured alternately with other fungicides, there was decrease in the development of Carbendazim resistance as compare to Continuous use. When *Alternaria alternata* (Fr.) Keissl. cultured alternately with other fungicide, there was decrease in Carbendazim resistance. Carbendazim when used alternately with Aliette, Ridomil, Roko, Curzate, there was complete inhibition of the growth of pathogen at 3rd and 4th passages. For mixed passage, it was observed that Carbendazim in mixture with Aliette completely inhibited the growth of pathogen at second passage, followed by Ridomil, Roko, Curzate at 3rd and 4th passages respectively.

Index Terms - Carbendazim, Passage, *Alternaria alternata* (Fr.) Keissl., Blight of chickpea.

I. INTRODUCTION

Pulses are an important part of the daily diet for most Indians as they contain 2 to 3 times more protein than cereals. Chickpea (*Cicer arietinum* L.) is the most important familiar pulse food crop and extensively cultivated in Mediterranean and Indian subcontinent regions of the world. In India, it is widely cultivated as a winter (rabi) crop and it belongs to family Leguminosae. The major regions where chickpea is cultivated in India are Maharashtra, Uttar Pradesh, Karnataka, Haryana, West Bengal, Gujarat, Bihar, and Chhattisgarh.

The chickpea plant is infected by *Alternaria alternata* (Fr.) Keissl. and causes alternaria blight disease showing severe damage (Mishra, 2005). Survey of pathogens infecting chickpea from 14 different localities of Maharashtra was carried out followed by MIC (Minimum Inhibitory Concentration) using systemic fungicide Carbendazim (Bavistin).

Alternaria alternata (Fr.) Keissl. causing leaf blight of chickpea is severe in all pathogens infecting chickpea. Carbendazim is recommended for the management of chickpea diseases. Hence, this fungicide was undertaken for detailed study. Reports on fungicide resistance in plant pathogens of various crops plants are very few. Day by day, there is increasing use of fungicides in management of diseases. The aim of the present investigation was therefore, to examine the possibility of development of resistance during passage in *Alternaria alternata* (Fr.) Keissl. against Carbendazim.

II. MATERIAL AND METHODS

Infected samples of chickpea leaf blight were collected and brought to the laboratory in clean sterilized polythene bags from 14 different localities of Maharashtra state. The pathogen (*Alternaria alternata* (Fr.) Keissl.) was isolated by direct plating analysis (Pitt and Hocking, 1997) and inoculated on Czapek Dox agar medium. The plates were incubated for 12 days and then screened. The isolated pathogens were identified (Subramanian, 1971; Barnett and Hunter, 1972). The pathogenicity test of which was confirmed by Koch's postulate. During investigation, it was found that *Alternaria alternata* (Fr.) Keissl. was found severe in all pathogens infected chickpea. Carbendazim was used to manage the disease. Highly resistant and sensitive isolates were identified and numbered.

Aa-1 to Aa-14 samples were checked (Aa - 1 - wild sensitive with 10 µg/ml and Aa- 11-highly resistant 400 µg/ml) using MIC of Carbendazim on agar plate (*in vitro*) and *in vivo* on chickpea plant. After determining MIC (wild sensitive isolates - Aa-1), the effect was examined during continuous, alternate and mixed passage treatments of Carbendazim and other fungicides having different modes of action on the development of resistance in the wild sensitive isolates of *Alternaria alternata* (Fr.) Keissl. on agar plates (*in vitro*) and on inoculated chickpea plants (*in vivo*).

In order to study the effect of passage *in vitro*, wild sensitive isolate (Aa-1) in each passage was cultured on agar plates containing a sub lethal dose of Carbendazim (10 µg/ml). The plates without fungicide served as Control. Inoculum from the colony of previous passage of the same isolate was placed at the center of each plate. In each passage linear growth was measured after 10 days. The percentage in increase of growth of the isolate from passage to passage was considered as increase in

Carbendazim resistance. The development of resistance was studied up to 8th passage. Continuous, as well as alternate passage of Carbendazim with Aliette, Ridomil, Roko, Curzate and mixed passage with the same fungicides were carried out.

In vivo studies were carried out on the chickpea plants. Leaves were dipped in the solution of Carbendazim alone, alternate and in mixture with other fungicides. They were inoculated with the wild sensitive isolate. An infected leaf portion of 1st passage was used for re-isolation and used as the source of inoculums for 2nd passage. This was continuously carried out up to the last passage. After 10 days of inoculation, the percentage of infection was recorded after incubation periods.

A) *In Vitro* studies

1. Continuous Passage

To study the effect of continuous passage, on Carbendazim resistance in *Alternaria alternata* (Fr.) Keissl. (i*n vitro),* wild sensitive isolate (Aa-1) in each passage was cultured on plates containing Carbendazim alone (10ppm) in triplicate. 8 mm diameter agar disc from the previous passage of the same isolate was placed at the centre of each plate in triplicate. In each passage, linear mycelial growth was measured after 10 days.

2. Alternate Passage

To study the effect of alternate passage on Carbendazim resistance, *Alternaria alternata* (Fr.) Keissl. isolate was cultured on plates containing Carbendazim, in triplicates. After 10 days, 8 mm diameter agar disc from the previous passage was transferred to the plates containing other fungicides alternately, at the same concentration. The process of such alteration of Carbendazim to other fungicides was continued up to 8th passage.

3. Mixed passage

To study the effect of mixed passage on Carbendazim resistance in *Alternaria alternata* (Fr.) Keissl. isolate was cultured on plates containing Carbendazim with another fungicide, both having equal concentration, in triplicates. After 10 days, 8 mm diameter agar disc from the previous passage was transferred to the plates containing the same mixture of fungicides, in equal concentration. In each type of passage, mentioned above, the increased mycelial growth from passage to passage was considered as criterion for the development of fungicide resistance. The effect of passage on the development of fungicide resistance in the pathogen was studied up to 8th passage, in each case.

B) *In vivo* studies

1. Continuous Passage

To study the effect of continuous passage, on the development of fungicide resistance in the pathogen (*in vivo*), mycelial suspension using one culture tube of wild sensitive isolate (Aa-1) was prepared. 30 ml mycelial suspension was inoculated on the fenugreek roots, treated with 5 ppm Carbendazim before 24 hrs. After 12 days a mycelial suspension from such infected roots was prepared and applied to healthy plant in the triplicate of chickpea treated with 5 ppm Carbendazim, 24 hrs before inoculation. Same procedure was followed up to 8th passage.

2. Alternate Passage

To study the effect of alternate passage, on the development of fungicide resistance, in the pathogen, *in vivo*, 30 ml mycelial suspension using 1 culture tube of wild sensitive isolate (Aa-1) was inoculated on the healthy plant in the triplicate of chickpea, treated with 5 ppm Carbendazim 24 hrs before it. After 8 days a mycelial suspension from such infected leafs was prepared and applied to healthy plant in triplicate of chickpea treated with another fungicide. Same procedure was followed up to 8^{th} passages alternately.

3. Mixed Passage

To study the effect of mixed passage, on the development of fungicide resistance, in the pathogen (in vivo), mycelial suspension using 1 culture tube of wild sensitive isolate (Aa-1) was inoculated on the healthy plant, treated with mixture of Carbendazim and another fungicide in same proportion, 24hrs before it. After 12 days a mycelial suspension from such infected leaves was prepared and applied to healthy plants in the triplicate treated with same mixture of fungicides, in same concentration before 24 hrs. In all above types of passages the chickpea plants were covered with polythene bags.

III. RESULTS

Table 1 - Effect of exposure of *Alternaria alternata* (Fr.) Keissl. to Carbendazim continuous and alternating with other fungicides on the development of Carbendazim resistance during eight successive passages. (*In vitro*)

Fungicides 10 µg/ml	**Passage number**							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Carbendazim Continuous	09.66	11.33	13.66	14.33	16.33	17.66	19.33	21.66
Carbendazim alters Aliette	10.33	12.66	00.00	00.00	00.00	00.00	00.00	00.00
Carbendazim alters Ridomil	10.66	11.33	13.66	00.00	00.00	00.00	00.00	00.00
Carbendazim alters Roko	11.33	10.33	00.00	00.00	00.00	00.00	00.00	00.00
Carbendazim Alters Curzate	11.66	16.33	11.33	00.00	00.00	00.00	00.00	00.00

Table 2 - Effect of exposure of *Alternaria alternata* (Fr.) Keissl. to the mixture of Carbendazim with other fungicides on the development of Carbendazim resistance during eight successive passages. (In vitro)

Fungicides 10µg/ml	Passage number							
	1	2	3	4	5	6	7	8
Carbendazim + Aliette	09.33	00.00	00.00	00.00	00.00	00.00	00.00	00.00
Carbendazim + Ridomil	10.33	09.66	00.00	00.00	00.00	00.00	00.00	00.00
Carbendazim +Roko	09.66	10.33	10.66	00.00	00.00	00.00	00.00	00.00
Carbendazim +Curzate	10.66	09.33	09.66	00.00	00.00	00.00	00.00	00.00

Table 3 - Effect of exposure of Alternaria *alternata* (Fr.) Keissl. alternata to Carbendazim continuous and alternating with other fungicides on the development of Carbendazim resistance during eight successive passages. (In vivo)

Fungicides 5 µg/ml		Passage number							
		1	2	3	4	5	6	7	8
Carbendazim Continuous	Percentage of infection	20.00	20.00	40.00	60.00	60.00	60.00	80.00	80.00
Carbendazim alters Aliette	Percentage of infection	20.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
Carbendazim alters Ridomil	Percentage of infection	20.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
Carbendazim alters Roko	Percentage of infection	20.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
Carbendazim Alters Curzate	Percentage of infection	20.00	20.00	00.00	00.00	00.00	00.00	00.00	00.00

Table 4 - Effect of exposure of Alternaria *alternata* (Fr.) Keissl. to the mixture of Carbendazim with other fungicides on the development of Carbendazim resistance during eight successive passages.(In vivo)

Fungicides 2 µg/ml	Passage number								
		1	2	3	4	5	6	7	8
Carbendazim + Aliette	Percentage of infection	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
Carbendazim + Ridomil	Percentage of infection	20.00	20.00	00.00	00.00	00.00	00.00	00.00	00.00
Carbendazim + Roko	Percentage of infection	20.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
Carbendazim + Curzate	Percentage of infection	20.00	20.00	00.00	00.00	00.00	00.00	00.00	00.00

A) *In vitro* studies

It was seen that growing of *Alternaria alternata* (Fr.) Keissl. on the medium containing Carbendazim for eight successive passages continuously, increased the resistance constantly till 8th passage. When it was cultured alternately with other fungicides, there was decrease in the development of Carbendazim resistance as compare to continuous use. It was observed that when *Alternaria alternata* (Fr.) Keissl. cultured alternately with other fungicide, there was decrease in Carbendazim resistance, Carbendazim when used alternately with Aliette, Ridomil, Roko, Curzate, there was complete inhibition of the growth of pathogen at 3rd and 4th passages. For mixed passage, it was observed that Carbendazim in mixture with Aliette completely inhibited the growth of pathogen at 2nd passage, followed by Ridomil, Roko, Curzate at 3rd and 4th passages respectively.

B) In vivo studies

For continuous passage in field it was observed that due to continuous treatment of Carbendazim there is increase in Carbendazim resistance in *Alternaria alternata* (Fr.) Keissl. significantly. For alternate passage, it was observed that Carbendazim when altered with Aliette, Ridomil and Roko completely inhibited infection on 2nd passage only. For mixed passage Carbendazim with Aliette was best combination as it completely inhibits infection at 1st passage only.

IV. DISCUSSION

Carbendazim is as a systemic fungicide used for management of many fungal diseases. Treatment of Carbendazim continuously, alternately and in mixture show variable results during eight passages *in vitro* and *in vivo*. These results are agreeing with other workers, regarding resistance of pathogen and application of fungicide and its mode of action on pathogen. Horsten (1979), observed that alternate use of Roko with Carbendazim reduced Carbendazim resistance in *Septoria nodorum* and *Cercosporella herpotrichoides.* Kable and Jaffery (1980), gave a mathematical model to test different chemicals for their alternate use. Anitha *et. al.*, (1989) also supported this work in Macrophomina phaseolina for

acquired and cross fungicide tolerance to Carbendazim. Gangawane *et. al*., (1995) studied the effect of passage on the development of metalaxyl resistance in *Phytophthora infestans*. Desai *et. al.* (2016) reported the effect of passage on the development of thiophonate methyl resistance in *Penicillium digitatum*. Hartill (1983), advised alternate use of mancozeb with metalaxyl to control late blight of potatoes. Again he observed development of Carbendazim resistance in *Macrophomina phaseolina* mutants having resistance factor from 5-15, mutants having high resistant factor exhibition higher growth and all mutants were stable for Carbendazim resistance. This might happen due to change in target sites. According to Waghmare and Andoji (2021), alternately used fungicide must have different mode of action.

V. ACKNOWLEDGMENT

The authors are thankful to the Principal of The New College, Kolhapur.

REFERENCES

Anitha, R., Reddy, M. R. and Rao, K. C. 1989. Studies on acquired fungicide tolerance in *Macrophomina phaseolina* (Tassi) Goid. to Mancozeb and Captan, and their cross tolerance to other fungicides. Ind. J. of Plant protection, 17(1):155-158.

Barnett, H. L. and Hunter, B. B. 1972. Illustrated Genera of Imperfect Fungi. Burgess Publishing Co. Minneapolis, Mn, USA, 241 pp.

Desai, U. A., Andoji Y.S and Kamble S.S. 2016. Influence of temperature and different culture media on growth of *Fusarium udum* (Butler) causal organism of wilt of pigeon pea. Int. J Biol.Res.,4 (1): 42-45.

Gangawane, L. V. and Kamble, S. S. and Arora, R. K. (1995). Synergistic effect of fungicides on metalaxyl resistant isolates of *Phytophthora infestans* from Nilgiri Hills. *Indian J. Plant Prot*. 23: 159-162.

Hartill, W. F. T. 1983. Development in New Zealand of resistance to dicarboximides fungicides in *Botrytis cinerea* to acylalanines in *Phytophthora infestans* and guazatine in *Penicillium italicum.* New Zealand J.Agril.Research.26*:*261-269, 1983.

Horsten, J. A. H. M. (1979). Acquired resistance to systemic fungicide of *Septoria nodorum* and *Cercosporella herpotrichoides* in cereals. Ph. D. Dissertation, Wageningen Uni., Netherlands, pp. 107.

Kable, P. F. and Jaffery, H. (1980). Selection for tolerance in organism exposed to sprays of biocide mixtures a theoretical model", Phytopathol, 70: 8- 12, 1980.

Mishra, V. 2005. Donor for resistance in Chickpea against *Alternaria alternata* (Fr.) Keisslar). J. Phytol. Res. 18(2): 265-266.

Pitt, J. I. and Hocking, A. D. 1997. Fungi and food spoilage, Second Edn. Blackie Academic and Professional, London.

Subramanian, C. V. 1971. *Hyphomycetes*, Indian Council of Agricultural Research, New Delhi, India. 930 pp.

Waghmare, V. U. and Andoji, Y. S. 2021. Biochemical changes in Benomyl sensitive and resistant isolates of *Fusarium solani* (Mart.) sacc. causing root rot of chickpea (*Cicer arietinum* L.).WJPR, 2021: 10 (9): 1005-1013.