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Studies on chemical mediated induction of resistance in chickpea against dry root rot (*Rhizoctonia bataticola*)

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Abstract
The present research work on, "Investigations on chemical mediated induction of resistance in chickpea against dry root rot (*Rhizoctonia bataticola*)." was carried out in the Section of Plant Pathology, College of Agriculture, Indore, during 2011-12 using variety JG-62. The object of the present investigation were evaluation of SAR inducing chemicals against *Rhizoctonia bataticola in vitro*, evaluation of SAR inducing chemicals for control of dry root rot in poly house and for control of dry root rot in the field at different concentrations. The experiments were conducted with nine treatments in a Randomized Block Design with three replications and Completely Randomized Design with four replications; observations were recorded at different days after sowing. The typical symptoms of the disease and characterization of the pathogen were described to identify it as *R. bataticola*. Studies on control of dry root rot by seed treatments and foliar application of SAR compounds were undertaken. Among the tested treatments salicylic acid at 400ppm applied as seed treatment along with its foliar application at 30 DAS and seed treatment with 200ppm isonicotinamide and foliar application of isonicotinamide with 200ppm at 30 DAS were most effective. Seed treatments with 200ppm salicylic acid with 200ppm isonicotinamide was the least effective treatment against *R. bataticola.* Experiments carried out to evaluate the resistance through salicylic acid and isonicotinamide reduced disease incidence caused by *R. bataticola* by imparting resistance to the host rather than directly affecting the pathogen. Studies on effect of seed treatments and foliar application on the incidence of dry root rot in chickpea showed that the disease incidence was significantly reduced by combination seed treatment with 400ppm salicylic acid and foliar application of salicylic acid with 400ppm at 30 DAS and seed treatment with 200ppm isonicotinamide and foliar application of isonicotinamide with 200ppm at 30 DAS. But salicylic acid at 400ppm applied as seed treatment along with its foliar application at 30 DAS proved as the best with respect to other control measures.

Keywords: Dry root rot, seed treatment, foliar application, SAR inducing chemicals and yield

Introduction
Chickpea (*Cicer arientimum* L.) is one of the most important pulse crops grown in the semi arid and tropical climate. There are two main types as desi (small in size having brown colour) and kabuli (bold seeded with light brown colour). It is a rich source of protein, vitamins and minerals containing 17-22% protein, 60-64% carbohydrate and 3-4% fat alongwith a good source of phosphorus and calcium. Chickpea leaves contain malic acid which bear medicinal value in intestinal disorders.

India grows chickpea on about 8.56 million ha area producing 7.35 million tonnes, which represents 38% and 50% of national pulse acreage and production, respectively. Chickpea production has gone up from 3.65 to 7.35 million tones and area has also increased from 7.57 to 8.56 million ha and the productivity has steadily increased to 858 kg/ha from 482 kg/ha. Notwithstanding its distribution throughout the country, six states *viz.,* Madhya Pradesh, Rajasthan, Maharashtra, Uttar Pradesh, Karnataka and Andhra Pradesh together contribute 92% of the production and 95% of the area of the country.

Major losses in chickpea yield are attributed mainly due to soil borne pathogens. The crop suffers from a number of soil borne pathogens like dry root rot (*Rhizoctonia bataticola*), collar rot (*Sclerotium rolfsii)* and wilt (*Fusarium oxysporum).* In recent years, induced resistance following treatment with biotic and abiotic factors has been considered to be a potential approach for the control of plant diseases. Therefore, induced resistance holds promise as a new technology for the control of plant diseases and has been proven to be effective in the laboratory and in a few field cases. Resistance inducing chemicals that are able to induce broad spectrum of disease resistance, offer an additional option for the farmers to complement with genetic disease resistance. *Rhizoctonia bataticola* lacks fruiting bodies and spores.

The mycelium is light-brown, thick in which black sclerotia are formed. Sclerotia are variable in form, small and loosely connected by mycelial threads. The fungus is soil-borne and may survive in the soil in the form of sclerotia for long time. The fungus has a wide host range and can infect the roots of several crops. Therefore, crop rotation is not effective to control the disease.
Various chemicals have been discovered that seem to act at various points, these defense activating networks mimic all or part of the biological activation of resistance. In the present investigation an attempt was made to correlate the appearance of wilt in different treatment of chemical SAR compounds.

Materials and Methods

The present studies on "chemicals mediated induction of resistance in chickpea against dry root rot *(Rhizoctonia* bataticola)." As SAR/ISR compounds and response of these chemicals to different inoculum load and substratum were carried out in the laboratory, under pot condition and in field experiments in the Department of Plant Pathology, College of Agriculture Indore, M.P. A trial to evaluate the effect of two SAR chemicals (salicylic acid and isonicotinamide) against the control of *R. bataticola* infection under natural condition in disease prone area was conducted. A trial was comprising nine treatments with three replications and randomized block design.
The per cent disease incidence was calculated by using the following formula.

$$\text{Per cent disease incidence} = \frac{\text{Number of plants affected}}{\text{Total number of plants observed}} \times 100$$

Test organism/ Isolation and purification of the pathogen

The pathogen *Rhizoctonia bataticola* was isolated from root region of the diseased plants by time segment method (Rangaswamy, 1972) and purified by hyphal tip method and maintained on potato dextrose agar stants. The affected portions (roots) of the diseased plants were cut with the help of sharp razor and rinsed with sterilised water to remove traces of dirt. These were surface sterilised by dipping in 1:1000 murcuric chloride solution for one minute and washed twice with sterile water. These pieces were transferred aseptically to sterilised Petri dishes containing solidified PDA-s in a laminar air flow. The Petri dishes were incubated at 26±1ºC. The appearing fungus was observed after 72 hours and isolations were made from developing colonies for further study.

Rhizoctonia bataticola

The morphological, cultural and formation of sclerotia were the principle characters to identify the pure cultures of *R. bataticola.* The characters were compared with those described by Ashby (1927) and the fungus was identified as *R. bataticola.*

Multiplication of fungal inoculums

The fungal inoculum was multiplied on sterilized sand maize meal medium in 1000 ml flasks containing 400 g in each flask. A loop full of fungus from actively growing culture was inoculated into each flask and incubated at room temperature for 15 days. The fungal culture multiplied on sand maize meal medium was used for inoculation to the soil.

Results and Discussion

Incidences of dry root rot at 30, 60 and 90 DAS

The minimum disease incidence (8.25) was observed in application of seed treatment with 400ppm salicylic acid and foliar application of salicylic acid with 400ppm at 30 DAS which was statistically identical to the incidence in seed treatment with 200ppm isonicotinamide and foliar application of isonicotinamide with 200ppm at 30 DAS (9.99). The minimum disease incidence (15.37) was observed in application of seed treatment with 400ppm salicylic acid and foliar application of salicylic acid with 400ppm at 60 DAS which was statistically identical to the incidence in seed treatment with 200ppm isonicotinamide and foliar application of isonicotinamide with 200ppm at 30 DAS (15.68), seed treatment with 200ppm salicylic acid (17.29) and seed treatment with 200ppm salicylic acid and foliar application of salicylic acid with 200ppm at 60 DAS (17.72). The minimum disease incidence (23.44) was recorded in seed treatment with 400ppm salicylic acid and foliar application of salicylic acid with 400ppm at 30 DAS which was statistically identical to the incidence in seed treatment with 200ppm isonicotinamide and foliar application of isonicotinamide with 200ppm at 90 DAS (25.11), seed treatment with 200ppm salicylic acid and foliar application of salicylic acid with 200ppm at 30 DAS (25.88), and seed treatment with 400ppm isonicotinamide and foliar application of isonicotinamide with 400ppm at 90 DAS (27.40).
The next effective treatment was seed treatment with 200ppm isonicotinamide and foliar application of isonicotinamide with 200ppm at 30 DAS. (9.99), (15.68) and (25.11) recorded at 30 DAS, 60 DAS and 90 DAS, respectively.

Table 1: Incidence of dry root rot in chickpea under the influence of chemical elicitors

Treatments	Treatment Description	Mean incidence of dry root rot (%)		
		30 DAS	60 DAS	90 DAS
T1	ST with 200ppm SA	6.15 (14.32)*	12.56 (20.74)	27.20 (31.44)
T2	ST with 400ppm SA	5.12 (13.07)	8.89 (17.29)	23.05 (28.66)
T3	ST with 200ppm INT	6.02 (14.07)	12.27 (20.47)	31.15 (33.89)
T4	ST with 400ppm INT	6.12 (14.30)	11.09 (19.44)	28.17 (32.03)
T5	ST with 200ppm SA and FS of SA with 200ppm at 30 DAS	4.07 (11.60)	9.27 (17.72)	19.08 (25.88)
T6	ST with 400ppm SA and FS of SA with 400ppm at 30 DAS	2.07 (8.25)	7.15 (15.37)	16.17 (23.44)
T7	ST with 200ppm INT and FS of INT with 200ppm at 30 DAS	3.05 (9.99)	7.31 (15.68)	18.01 (25.11)
T8	ST with 400ppm INT and FS of INT with 400ppm at 30 DAS	4.16 (11.75)	10.31 (18.67)	21.28 (27.40)
T9	Control	8.14 (16.55)	30.03 (33.21)	50.01 (45.01)
	S.Em±	0.81	0.91	1.52
	CD	2.43	2.74	4.57

* The data transformed by Angular transformation.

Influence of chemical elicitors on germination percent of chickpea at 10 DAS

The study of the data revealed that the minimum germination percent (43.62) was found in control which was statistically identical to seed treatment with 200ppm isonicotinamide (45.17), seed treatment with 200ppm salicylic acid (47.93), seed treatment with 400ppm salicylic acid (51.71), seed treatment with 400ppm isonicotinamide and foliar application of isonicotinamide with 400ppm at 30 DAS (51.95), seed treatment with 400ppm isonicotinamide (52.30) and seed treatment with 200ppm salicylic acid and foliar application of salicylic acid with 200ppm at 30 DAS (53.35). The maximum germination percent (55.60) was found in seed treatment with 400ppm salicylic acid and foliar application of salicylic acid with 400ppm at 30 DAS which was statistically at par with the earlier set of seed treatment with 200ppm salicylic acid (47.93), seed treatment with 400ppm salicylic acid (51.71), seed treatment with 400ppm isonicotinamide and foliar application of isonicotinamide with 400ppm at 30 DAS (51.95), seed treatment with 400ppm isonicotinamide (52.30) seed treatment with 200ppm salicylic acid and foliar application of salicylic acid with 200ppm at 30 DAS (53.35) and seed treatment with 200ppm isonicotinamide and foliar application of isonicotinamide with 200ppm at 30 DAS (55.43).

Table 2: Influence of chemical elicitors on germination percent of chickpea at 10 DAS

Treatments	Treatment Description	Germination (%) 10 DAS
T1	ST with 200ppm SA	55.09 (47.93) *
T2	ST with 400ppm SA	61.52 (51.71)
T3	ST with 200ppm INT	50.29 (45.17)
T4	ST with 400ppm INT	62.46 (52.30)
T5	ST with 200ppm SA and FS of SA with 200ppm at 30 DAS	63.51 (53.35)
T6	ST with 400ppm SA and FS of SA with 400ppm at 30 DAS	67.95 (55.60)
T7	ST with 200ppm INT and FS of INT with 200ppm at 30 DAS	67.72 (55.43)
T8	ST with 400ppm INT and FS of INT with 400ppm at 30 DAS	61.99 (51.95)
T9	Control	47.60 (43.62)
	S.Em±	3.29
	CD	9.86

*The data transformed by Angular transformation.

Radial growth of *Rhizoctonia bataticola* as influenced by chemical elicitors

The data on colony diameter was recorded at 7 days of incubation. The fungus grew in its characteristics manner in all the treatments (Table 3). The minimum radial growth (3.46) was recorded in 1000ppm salicylic acid. The next higher radial growth (4.48) was recorded in 200ppm isonicotinamide (Plate No. 2 and 3). The next higher radial growth (6.75) was observed in 1000ppm isonicotinamide followed by 600ppm salicylic acid (6.94), control (7.15) 600ppm isonicotinamide (7.45) which were statistically at par. Still the next higher radial growth (6.94) was recorded in 600ppm salicylic acid followed by control (7.15), 600ppm isonicotinamide (7.45) and 400ppm salicylic acid (7.84). The maximum radial growth was recorded in 400ppm isonicotinamide (8.43) which was statistically at par with earlier set of 400ppm salicylic acid (7.84) and 200ppm salicylic acid (8.38).

Table 3: Radial growth of *Rhizoctonia bataticola* as influenced by chemical elicitors.

Treatments	Treatments description	Radial growth of *R. bataticola* (cm) after 7 days
T1	200ppm salicylic acid	8.38
T2	400ppm salicylic acid	7.84
T3	600ppm salicylic acid	6.94
T4	1000ppm salicylic acid	3.46
T5	200ppm isonicotinamide	4.48
T6	400ppm isonicotinamide	8.43
T7	600ppm isonicotinamide	7.45
T8	1000ppm isonicotinamide	6.75
T9	Control	7.15
	S.Em±	0.32
	CD	0.92

Height of chickpea plants as influenced by chemical elicitors

The minimum plant height (39.20) was recorded in control followed by seed treatment with 400ppm isonicotinamide (39.27), seed treatment with 200ppm salicylic acid and foliar application of salicylic acid with 200ppm at 30 DAS (39.27), seed treatment with 200ppm isonicotinamide (39.47), seed treatment with 400ppm salicylic acid (40.40), seed treatment with 400ppm isonicotinamide and foliar application of isonicotinamide with 400ppm at 30 DAS (40.47), seed treatment with 200ppm salicylic acid (40.60), seed treatment with 200ppm isonicotinamide and foliar application of isonicotinamide with 200ppm at 30 DAS (40.60), and seed treatment with 400ppm salicylic acid and foliar application of salicylic acid with 400ppm at 30 DAS (40.73). Thus the data revealed that there were statistically non-significant difference between all the treatments but the seed treatment with salicylic acid 200ppm resulted in maximum of plant.

Table 4: Height of chickpea plants as influenced by chemical elicitors

Treatments	Treatment description	Plant height (cm)
T1	ST with 200ppm SA	40.60
T2	ST with 400ppm SA	40.40
T3	ST with 200ppm INT	39.47
T4	ST with 400ppm INT	39.27
T5	ST with 200ppm SA and FS of SA with 200ppm at 30 DAS	39.27
T6	ST with 400ppm SA and FS of SA with 400ppm at 30 DAS	40.73
T7	ST with 200ppm INT and FS of INT with 200ppm at 30 DAS	40.60
T8	ST with 400ppm INT and FS of INT with 400ppm at 30 DAS	40.47
T9	Control	39.20
	S.Em±	1.14
	CD	NS

Number of branches per plants as influenced by chemical elicitors

The data indicated that the minimum number of branches (10.87) was observed in control and seed treatment with 200ppm salicylic acid and foliar application of salicylic acid with 200ppm at 30 DAS (10.87) which was statistically identical to treatment with 400ppm salicylic acid (10.93), seed treatment with 200ppm isonicotinamide and foliar application of isonicotinamide with 200ppm at 30 DAS (11.13), seed treatment with 200ppm isonicotinamide and foliar application of isonicotinamide with 200ppm at 30 DAS (11.20), seed treatment with 200ppm isonicotinamide (11.27), seed treatment with 400ppm isonicotinamide (11.67). The maximum number of branches (12.07) was observed in seed treatment with 400ppm salicylic acid and foliar application of salicylic acid with 400ppm at 30 DAS which was statistically at par with the earlier set of seed treatment with 200ppm isonicotinamide (11.27), seed treatment with 400ppm isonicotinamide (11.67) and seed treatment with 200ppm salicylic acid (11.80).

Table 5: Number of branches per plant as influenced by chemical elicitors

Treatments	Treatment description	Number of branches/plant
T1	ST with 200ppm SA	11.80
T2	ST with 400ppm SA	11.67
T3	ST with 200ppm INT	11.27
T4	ST with 400ppm INT	11.67
T5	ST with 200ppm SA and FS of SA with 200ppm at 30 DAS	10.87
T6	ST with 400ppm SA and FS of SA with 400ppm at 30 DAS	12.07
T7	ST with 200ppm INT and FS of INT with 200ppm at 30 DAS	11.13
T8	ST with 400ppm INT and FS of INT with 400ppm at 30 DAS	11.20
T9	Control	10.87
	S.Em±	0.28
	CD	NS

Number of pods per plants as influenced by chemical elicitors

The minimum number of pods (49.87) was recorded in control which was statistically identical to seed treatment with 200ppm salicylic acid (51.73), seed treatment with 200ppm isonicotinamide (55.60), seed treatment with 400ppm isonicotinamide (56.13), seed treatment with 400ppm isonicotinamide and foliar application of isonicotinamide with 400ppm at 30 DAS (56.53), seed treatment with 200ppm salicylic acid and foliar application of salicylic acid with 200ppm at 30 DAS (57.47) and seed treatment with 400ppm salicylic acid (58.07). The higher number of pods was recorded in seed treatment with 200ppm salicylic acid (51.73) followed by seed treatment with 200ppm isonicotinamide (55.60), seed treatment with 400ppm isonicotinamide (56.13), seed treatment with 400ppm isonicotinamide and foliar application of isonicotinamide with 400ppm at 30 DAS (56.53), seed treatment with 200ppm salicylic acid and foliar application of salicylic acid with 200ppm at 30 DAS (57.47), seed treatment with 400ppm salicylic acid (58.07) and seed treatment with 200ppm isonicotinamide and foliar application of isonicotinamide with 200ppm at 30 DAS (58.60).

The maximum number of pods was recorded in seed treatment with 400ppm salicylic acid and foliar application of salicylic acid with 400ppm at 30 DAS (60.40) which was statistically higher than all the treatments except seed treatment with 200ppm isonicotinamide (55.60), seed treatment with 400ppm isonicotinamide (56.13), seed treatment with 400ppm isonicotinamide and foliar application of isonicotinamide with 400ppm at 30 DAS (56.53).

Table 6: Number of pods per plant as influenced by chemical elicitors

Treatments	Treatment description	No of pods per plant
T1	ST with 200ppm SA	51.73
T2	ST with 400ppm SA	58.07
T3	ST with 200ppm INT	55.60
T4	ST with 400ppm INT	56.13
T5	ST with 200ppm SA and FS of SA with 200ppm at 30 DAS	57.47
T6	ST with 400ppm SA and FS of SA with 400ppm at 30 DAS	60.40
T7	ST with 200ppm INT and FS of INT with 200ppm at 30 DAS	58.60
T8	ST with 400ppm INT and FS of INT with 400ppm at 30 DAS	56.53

T9	Control	49.87
	S.Em±	2.89
	CD	NS

Yield of chickpea in the influence of chemical elicitors

The minimum yield (10.22) was observed in control which was statistically identical to seed treatment with 400ppm isonicotinamide (10.56), seed treatment with 400ppm isonicotinamide and foliar application of isonicotinamide with 400ppm at 30 DAS (10.60), seed treatment with 200ppm salicylic acid (12.35), seed treatment with 200ppm isonicotinamide (13.87) and seed treatment with 200ppm salicylic acid and foliar application of salicylic acid with 200ppm at 30 DAS (14.54). The higher grain yield was recorded in seed treatment with 400ppm isonicotinamide (10.56) followed by seed treatment with 400ppm isonicotinamide and foliar application of isonicotinamide with 400ppm at 30 DAS (10.60), seed treatment with 200ppm salicylic acid (12.35), seed treatment with 200ppm isonicotinamide (13.87) and seed treatment with 200ppm salicylic acid and foliar application of salicylic acid with 200ppm at 30 DAS (14.54) and seed treatment with 400ppm salicylic acid (14.60).

The maximum grain yield was recorded in seed treatment with 400ppm salicylic acid and foliar application of salicylic acid with 400ppm at 30 DAS (15.86) which was statistically higher than all the treatments except seed treatment with 200ppm salicylic acid (12.35), seed treatment with 200ppm isonicotinamide (13.87) and seed treatment with 200ppm salicylic acid and foliar application of salicylic acid with 200ppm at 30 DAS (14.54) and seed treatment with 400ppm salicylic acid (14.60) and seed treatment with 200ppm isonicotinamide and foliar application of isonicotinamide with 200ppm at 30 DAS (15.25).

Table 7: Yield of chickpea in the influence of chemical elicitors

Treatments	Treatment description	Yield of chickpea (q/ha)
T1	ST with 200ppm SA	12.35
T2	ST with 400ppm SA	14.60
T3	ST with 200ppm INT	13.87
T4	ST with 400ppm INT	10.56
T5	ST with 200ppm SA and FS of SA with 200ppm at 30 DAS	14.54
T6	ST with 400ppm SA and FS of SA with 400ppm at 30 DAS	15.86
T7	ST with 200ppm INT and FS of INT with 200ppm at 30 DAS	15.25
T8	ST with 400ppm INT and FS of INT with 400ppm at 30 DAS	10.60
T9	Control	10.22
	S.Em±	1.44
	CD	NS

Conclusion

Studies on effect of seed treatments and foliar application on the incidence of dry root rot in chickpea showed that the disease incidence was significantly reduced by combination treatment seed treatment with 400ppm salicylic acid and foliar application of salicylic acid with 400ppm at 30 DAS, seed treatment with 400ppm isonicotinamide and foliar application of isonicotinamide with 400ppm at 30 DAS in field condition and salicylic acid at 400ppm applied as seed treatment along with its foliar application just after germination of the plants, isonicotinamide at 400ppm applied as seed treatment coupled with foliar application just after germination of the plants in pot condition were the best but salicylic acid at 400ppm applied as seed treatment along with its foliar application at 30 DAS was proved in best with respect to other control measures.

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