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# EFFICACY OF BIO-PESTICIDES AGAINST *SCLEROTIUM ROLFSII* SACC. CAUSING COLLAR ROT OF CHICKPEA (*CICER ARIETINUM* L.)

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**ABSTRACT**
In this study the bio efficacy of ten botanicals and eight antagonists was tested through poison food technique and dual culture technique respectively against *S. rolfsii* causing collar rot of chickpea. Among the 10 botanicals tested, cent per cent mycelial inhibition was observed in aqueous extract of *Agave* at different concentrations, followed by Henna leaves with 34.4, 71.3 and 90% at 5, 10 and 15 per cent concentration respectively and least mycelial inhibition was observed in *Tridax* leaves extract (5.5%) and *Pongamia* (7.1%). Among the eight bioagents tested against *S. rolfsii, Trichoderma harzianum*-55 IIHR recorded maximum inhibition of 70% followed by *T. harzianum* NBAII with 63% and least mycelial inhibition was observed in *Pseudomonas fluorescens* and *Bacillus subtilis*. Chickpea is one of important pulse crop which is infected with collar rot botanicals and bioagents can be used in IDM practice for management of this disease.

## INTRODUCTION

Chickpea is known in this country since ancient times. It is a widely grown major pulse crop in India, accounts for nearly 75 per cent of the total pulse production in the world. Chickpea crop is prone to many diseases *viz*., *Fusarium* wilt, dry root rot, collar rot, *Ascochyta* blight, *Verticillium* wilt, black root rot, *Phytophthora* root rot, wet root rot, foot rot, *Pythium* rot and seed rot etc. Among these, collar rot caused by *Sclerotium rolfsii* which is gaining importance. *Sclerotium rolfsii* is an economically important pathogen on numerous crops worldwide. It has an extensive host range; at least 500 species in 100 families are susceptible, the most common hosts are legumes, crucifers and cucurbits, and commonly occurs in the tropics, subtropics, and other warm temperate regions (Punja, 1985).

Management of soil borne plant pathogens including *Sclerotium rolfsii* can be achieved by different fungicides, soil fumigants (Methyl bromide) and bioagents. Frequent application of fungicides causes environmental pollution therefore there is a need to reduce the amount of chemicals applied to soil.

*Sclerotium rolfsii* has wide host range, abundant growth of the pathogen and its capability of producing excessive sclerotia that may persist in soil for several years (Chet and Henis, 1972; Punja, 1985). Hence management of *Sclerotium rolfsii* causing collar rot of chickpea is difficult to achieve chemically, In this context plant extracts and bioagents can be used as an alternative source for controlling soil-borne diseases since they comprise a rich source of bioactive substance (Wink, 1993). Plants extracts are eco-friendly possess protective, curative and antagonistic activity against many diseases. (Kandasamy *et al*., 1974; Hale and Mathers, 1977; Rahber-Bhatti, 1986; Kalo& Taniguchi, 1987). Biological control of plant diseases has been the subject of extensive research in the last two decades. *Trichoderma* spp. is well documented as effective biological control agents of plant diseases (Harman *et al*. 1980, Sivan *et al*., 1984 and Coley-Smith *et al*., 1991).

Therefore the present investigation was carried out to evaluate the bio efficacy of botanicals and antagonists against *S. rolfsii* causing collar rot of chickpea.

## MATERIALS AND METHODS

### *In vitro* evaluation of botanicals against *S. rolfsii*

The bioefficacy of the ten botanicals (Table1) were evaluated against *S. rolfsii*.

### Preparation of cold aqueous extract

Fresh sample of each test plant were collected and washed in tap water and then in distilled water. The aqueous extracts of botanicals were prepared by crushing fresh samples (100g) in a sterilized Pestle and Mortar by adding 100 ml sterile distilled water (1:1 w/v). The extract was used as stock solution. To study antifungal mechanism of plant extracts, poison food technique was followed as suggested by Nene and Thapliyal

(1982). The anti-fungal activity of plant extract was tested at 5, 10 and 15 per cent concentrations. The experiment was conducted in Completely Randomized Design (CRD) with three replication five, ten and fifteen ml of stock solution was mixed with 95, 90 and 85 ml of sterilized molten potato dextrose agar medium respectively. The medium was thoroughly shaken for uniform mixing of the extract. Twenty ml of medium was poured into each of the 90 mm sterilized Petri plates. The control was maintained without plant extracts. After solidification, each plate was inoculated with 5 mm mycelial disc taken from the periphery of seven day old fungal culture and incubated at 27±1°C. The observations were taken on the day when the growth of colony touched the periphery in the control. The per cent inhibition of mycelial growth over control was calculated by using the formula given by Vincent (1947) and the data analyzed statistically.

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

Where, I = per cent inhibition

C = growth in control

T = growth in treatment

***In vitro* evaluation of bioagents against *S. rolfsii***

*In vitro* evaluation of eight bioagents *viz.*, *Trichoderma harzianum* (Biocontrol lab, GKVK), *Trichoderma harzianum* (NBAII), *Trichoderma harzianum* (IIHR), *Trichoderma harzianum*-55 (IIHR), *Trichoderma viride* (Biocontrol lab, GKVK) *Trichoderma viride* (IIHR), *Pseudomonas fluorescens* (NBAII) and *Bacillus subtilis* (NBAII) against collar rot of chickpea causing pathogen (*S. rolfsii*) was carried out by dual culture technique (Morton and Strouble 1955).

Twenty ml of sterilized and cooled potato dextrose agar was poured into sterile Petri plates and allowed to solidify. The mycelial disc of test fungus was inoculated at one end and antagonistic fungus opposite to it. In case of evaluation of bacterial antagonist, the bacterium was streaked one day earlier at one end of the Petri plate and the test fungus was placed at the other end. The plates were incubated at 27±1ºC and zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and antagonistic organism. The colony diameter of pathogen in control plate was also recorded. The per cent inhibition of growth of the pathogen was calculated by using the formula suggested by Vincent (1947).

## RESULTS AND DISCUSSION

***In vitro* evaluation of botanicals**

Among the botanicals evaluated, *Agave* recorded maximum mycelial inhibition of 100 per cent at all the concentrations

**Table 1: List of botanicals evaluated against *S. rolfsii***

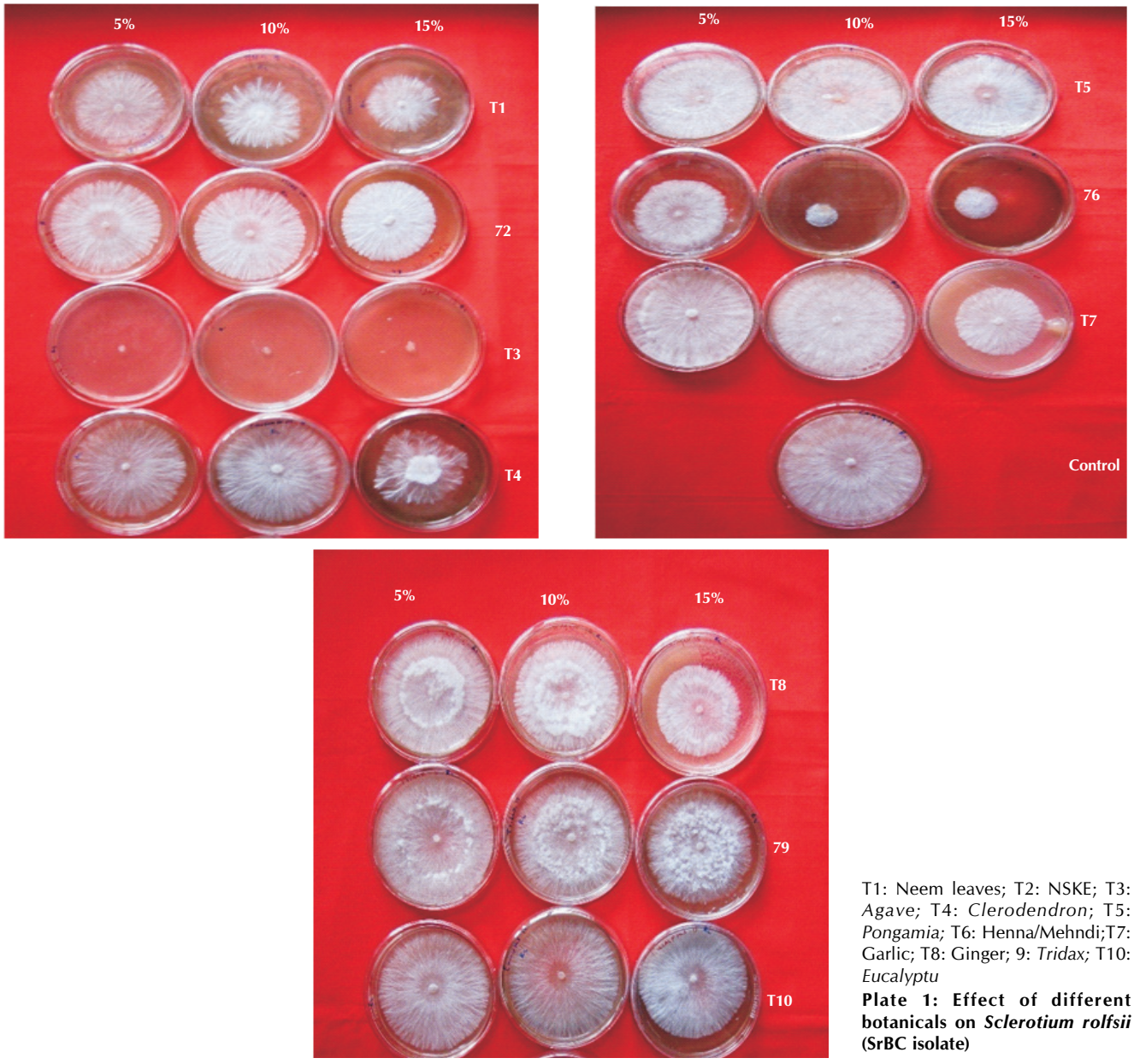
| # | Botanical name | Common name | Family | Part used |
|---|---|---|---|---|
| 1 | *Azadirachta indica*A. Juss. | Neem | Meliaceae | Leaves |
| 2 | *Azadirachta indica*A. Juss. | NSKE* | Meliaceae | Seed |
| 3 | *Agave tequilan*F.A.C. Weber | Agave | Asparagaceae | Leaves |
| 4 | *Clerodendrum inerme* (L.) Gaertn. | Glory flower | Lamiaceae | Leaves |
| 5 | *Pongamia pinnata* (L.) Panigrahi | Hongae tree | Leguminaceae | Leaves |
| 6 | *Lawsonia inermis* L. | Henna/Mehndi | Lythraceae | Leaves |
| 7 | *Allium sativum* L. | Garlic | Amaryllidaceae | Bulb |
| 8 | *Zingiber officinale* Roscoe. | Ginger | Zingiberaceae | Rhizome |
| 9 | *Tridax procumbens* L. | Coat buttons | Asteraceae | Leaves |
| 10 | *Eucalyptus oblique* L'Hér. | Eucalyptus | Myrtaceae | Leaves |

*NSKE- Neem seed kernel extract

**Table 2: *In vitro* evaluation of botanicals against *Sclerotium rolfsii* (SrBC isolate)**

| Sl. No. | Extract | Per cent mycelial inhibition Conc. of botanicals | | | |
|---|---|---|---|---|---|
| | | 5% | 10% | 15% | Mean |
| 1 | Neem leaves | 32.6(34.80)* | 41.1(39.86) | 41.3(39.97) | 38.3(38.24) |
| 2 | NSKE | 13.2(21.25) | 17.4(24.65) | 22.4(28.26) | 17.7(24.85) |
| 3 | *Agave* | 100.0(89.96) | 100.0(89.96) | 100.0(89.96) | 100.0(89.96) |
| 4 | *Clerodendron* | 13.2(21.25) | 18.6(25.50) | 28.9(32.49) | 20.2(26.69) |
| 5 | *Pongamia* | 5.9(14.09) | 5.4(13.39) | 9.9(18.31) | 7.1(15.40) |
| 6 | Henna | 34.4(35.92) | 71.3(57.58) | 90.0(71.54) | 65.2(53.86) |
| 7 | Garlic | 2.6(9.28) | 5.0(12.92) | 33.3(35.25) | 13.6(21.67) |
| 8 | Ginger | 5.2(13.16) | 12.8(20.93) | 30.4(33.47) | 16.1(23.67) |
| 9 | *Tridax* | 0 | 6.7(14.95) | 9.9(18.31) | 5.5(13.57) |
| 10 | *Eucalyptus* | 10.4(18.78) | 8.2(16.59) | 11.9(20.13) | 10.1(18.55) |
| 11 | Control | 0 | 0 | 0 | 0 |
| | Botanicals | Conc. | Botanicals X Concentration | | |
| SEm+ | 0.16 | 0.31 | 0.09 | | |
| CD (P0.01) | 0.52 | 0.99 | 0.30 | | |

*Figures in parentheses are Arcsine transformed values




T1: Neem leaves; T2: NSKE; T3: *Agave;* T4: *Clerodendron*; T5: *Pongamia;* T6: Henna/Mehndi;T7: Garlic; T8: Ginger; 9: *Tridax;* T10: *Eucalyptu*

**Plate 1: Effect of different botanicals on *Sclerotium rolfsii* (SrBC isolate)**

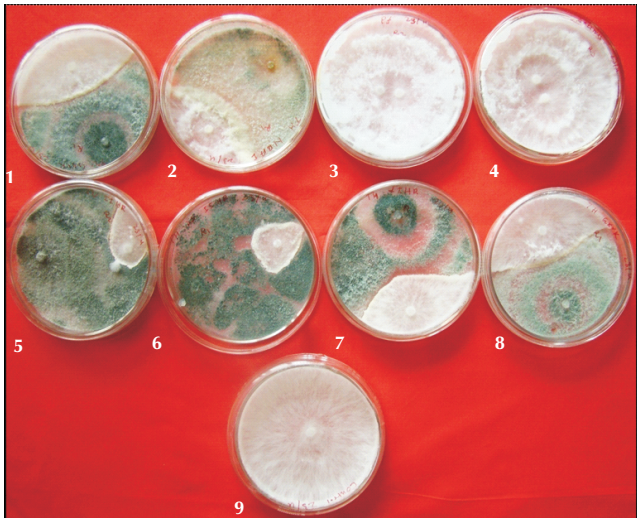
**Table 3: *In vitro* evaluation of bioagents against *Sclerotium rolfsii* (SrBC isolate)**

| Sl. No. | Bioagent | Per cent inhibition(Days After Incubation) 4 | 8 |
|---|---|---|---|
| 1 | *T. viride* GKVK | 36 (36.59)* | 36 (36.92) |
| 2 | *T. harzianum* NBAII | 49 (44.24) | 63 (52.31) |
| 3 | *P. fluorescens* NBAII | 0 | 0 |
| 4 | *B. subtilis* NBAII | 0 | 0 |
| 5 | *T. viride* IIHR | 59 (49.90) | 59 (50.09) |
| 6 | *T. harzianum*-55 IIHR | 66 (54.27) | 70 (56.66) |
| 7 | *T. harzianum* IIHR | 37 (37.71) | 37(37.69) |
| 8 | *T. harzianum* GKVK | 31(34.02) | 32(34.35) |
| 9 | Control | 0 | 0 |
| | SEm+ | 0.43 | 0.44 |
| | CD (P0.01) | 1.77 | 1.80 |

*Figures in parenthesis are arcsine transformed values

tested, followed by Henna leaves with 34.4, 71.3 and 90% at 5, 10 and 15 per cent concentration respectively with a mean of 65.25%. (Table 2, Plate 1) Neem extract recorded 32.6, 41.1 and 41.3 per cent inhibition at 5, 10 and 15 per cent concentrations with a mean of 38.3%; NSKE 13.2, 17.4 and 22.4 per cent at 5, 10 and 15 per cent concentrations with a mean of 17.7%; *Clerodendron* 13.2, 18.6 and 28.9 per cent at 5, 10 and 15 per cent concentrations with a mean of 20.19%; *Eucalyptus* 10.4, 8.2 and 11.9 per cent at 5, 10 and 15 per cent concentrations with a mean of 10.1%; Ginger 5.2, 12.8 and 30.4 per cent at 5, 10 and 15 per cent concentrations with a mean of 16.1%; Garlic 2.6, 5.0 and 33.3 per cent at 5, 10 and 15 per cent concentrations with a mean of 13.6% respectively showed moderate inhibition.

Least inhibition was observed in *Tridax* with 0.0, 6.7 and 9.9 per cent inhibition at 5, 10 and 15 per cent concentration




1. *Trichodermaviride* GKVK; 2. *Trichoderma harzianum* NBAII; 3. *Pseudomonas fluorescens;* 4. *Bacillus subtilis*; 5.*Trichoderma viride*IHHR; 6.*Trichoderma harzianum*-55 IIHR; 7.*Trichoderma harzianum* IHHR; 8.*Trichoderma harzianum* GKVK; 9. *S. rolfsii* (SrBC isolate)

**Plate 2: Mycelial inhibition by different bioagents in dual culture**

respectively with a mean of 5.5% and *Pongamia* recorded 5.9, 5.4 and 9.9 per cent inhibition at 5, 10 and 15 per cent concentration respectively with a mean of 7.1%.

The findings are in agreement with Seshakiran (2002), who observed that among the 30 plant extracts evaluated *in vitro, Agave americana* L. exhibited maximum inhibition of mycelial growth and sclerotial formation at 10 per cent concentration, Singh *et al***.** (2007) observed that neem extract (*Azadirachta indica*) caused the maximum inhibition of mycelial growth and sclerotial production, its size and viability whereas Kulkarni (2007) observed*Clerodendrum inerme* to show maximum inhibition of mycelial growth (53.33%). And also Sunita Mahapatra and Srikanta Das in 2013 Showed that three botanicals (Neem leaf extract, Ginger rhizome extract and Garlic bulb extract) significantly ($p < 0.05$) reduced the percent leaf infection *Alternaria* leaf blight of mustard in field conditions in comparison to untreated control. Sunaina Bisht *et al*., 2013 tested the plant extracts, essential oils against *Curvularia* leaf spot of maize they found that Lantana was highly effective @ 15 per cent (86.76 inhibition %) and 20 per cent (89.49 inhibition %) followed by Morphankhi @ 5 per cent (83.53 %) and 10 per cent (85.88%) respectively. Among the essential oils, complete inhibition was recorded in Citronella oil at all 3 concentrations (2ìL, 4ìL and 8) and Peppermint oil at 4ìL and 8 ìL concentrations and least inhibition was observed in Palmaroza (65 %) at 2ìL.

### *In vitro* evaluation of bioagents

After four days of incubation, maximum inhibition of mycelial growth (66%) was recorded in *T. harzianum*-55 IIHR that was superior over all other bioagents. *P. fluorescens* and *B. subtilis* did not show any inhibition of mycelial growth of *S. rolfsii* as the pathogen over grew the bioagents. Other bioagents *viz., T. viride* IIHR (59), *T. harzianum* NBAII (49), *T. harzianum* IIHR (37*), T. viride* GKVK (36) and *T. harzianum* GKVK (31) in that order showed moderate level of inhibition.

Similarly after 8 days of inoculation, the *T. harzianum*-55 IIHR showed highest inhibition (70%). Followed by *T. harzianum* NBAII (63%), *T. viride* IIHR (59%) whereas *T. harzianum* IIHR (37%), *T. viride* GKVK (36%) and *T. harzianum* GKVK (32%) did not show much variation in the inhibition percentage (Table 3, Plate,2).

Biological control is an effective, ecofriendly and alternative approach for management of any disease. All the species of *Trichoderma* showed more hyphal inhibition compared to bacterial antagonists. Kulkarni (2007), found maximum inhibition of mycelial growth in *T. harzianum* (Dharwad isolate) (59.81%), followed by *T. harzianum* of PDBC (57.97%) and least inhibition of mycelial growth was observed in *Bacillus subtilis* (10.74%). Similarly Basamma (2008) and Manu (2012) reported least inhibition by *B. subtilis* and *P. fluorescens* as against higher inhibition by *Trichoderma spp*. and Ritesh Kumar *et al.,* in 2012 studied the antagonistic potential of *Trichoderma* species isolated from two different soils *i.e* alfisols and inceptisols and they found that the alfisol isolates showed higher potential antagonism against *S. rolfsii* with percent inhibition of 44.67 and 47.88 as compared to inceptisol isolates with inhibition percentage of 3.97, 7.97 and 28.72 respectively. And compared to inceptisol isolates, biomass accumulation and total phenol content was also reported high in the alfisol isolates. Sunaina Bisht *et al*., 2013 tested different strains of *Trichoderma* spp. against *Curvularia* leaf spot of maize they found that *Trichoderma harzianum*, Th-13 shown maximum mycelial growth inhibition (83.82 %) followed by Th-9 (80.29 %) and Th-3 (79.12 %).

Dhingani *et al*., in 2013 tested the efficacy of botanicals and oil cakes against *Macrophomina phaseolina* (Tassi.)The extract of garlic cloves (*Allium sativum* L.) was proved excellent with maximum inhibiting (73 %) mycelial growth and sclerotial formation followed by rhizome extract of turmeric (*Curcuma longa* L) (63.98 %). The four organic extracts were tested against *M. phaseolina* by poisoned food technique *in vitro*. Significantly least growth of mycelium and maximum mycelium inhibition was recorded in extracts of neem cake (59.40 %) followed by farm yard manure (42.56 %). Next best in order of merit were castor cake and mustard cake.

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