**Bulletin of Environment, Pharmacology and Life Sciences**
Bull. Env. Pharmacol. Life Sci., Vol 6 Special issue [2] 2017: 412-417

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.533
Universal Impact Factor 0.9804
NAAS Rating 4.95


**FULL LENGTH ARTICLE** **OPEN ACCESS**

# In-*vitro* evaluation of Bioagents and chemical fungicides against dry root rot of chickpea caused by *Macrophomina phaseolina* in southern parts of Karnataka

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**ABSTRACT**

*A survey conducted for dry root rot in chickpea growing areas of southern parts of Karnataka showed that the disease incidence varied from 18.61 per cent to 61.24 per cent in different taluks. Among the districts, the highest mean disease incidence was recorded in Chitradurga (56.10 %) followed by Tumakuru (52.57 %), Bengaluru urban (51.13 %), Bengaluru rural (40.53 %), Chikkaballapur (36.41 %), Mysuru (32.09 %), Hassan (29.97 %) and Chikkamagaluru (24.73 %). In in-vitro evaluation all the antagonists significantly inhibited mycelial growth of M. phaseolina and per cent inhibition ranged from 62.96 to 81.44 per cent. Among fungal and bacterial antagonists, the maximum inhibition of mycelial growth was observed in Trichoderma harzianum (Th-55) isolate with 81.44 per cent and minimum inhibition was observed in T. harzianum (GKVK) with 62.96 per cent. Among five systemic fungicides evaluated, tebuconazole, carbendazim, difenoconazole and propiconazole recorded maximum mycelial inhibition of cent per cent at all concentrations. Whereas, thiophanate methyl recorded cent per cent inhibition at 500 ppm concentration. The contact fungicides viz., mancozeb, recorded cent per cent inhibition of mycelial growth at all the concentrations (250, 500, 750 and 1000 ppm) followed by thiram (80.10 %), captan (69.72 %) and chlorothalonil (62.22 %). The combi- products viz., carboxin 37.5 % + thiram 37.5 % WP and carbendazim 12 % + mancozeb 63 % WP showed cent per cent inhibition of mycelia at all concentrations (250, 500, 750 and 1000 ppm).*

***Key words:*** *Survey, In-vitro, Bio-agents, fungicides, Macrophomina phaseolina*

Received 12.07.2017 Revised 09.08.2017 Accepted 28.08.2017

## INTRODUCTION

Chickpea (*Cicer arietinum* L.), also known as Gram or Bengal gram, is the second most important pulse crop in the world, India accounting for 60 to 75 per cent of the world's chickpea production. Chickpea seeds contain high quality easily digestible protein (25 %) and carbohydrates (20 %) making it an important source of protein for the vegetarians of the country and thus it is also called "Poor man's meat." The origin of chickpea is thought to have been in South Eastern Turkey and neighbouring Northern Syria [21]. It has since spread to many other geographical regions of the world because of its ability to grow in diverse environmental conditions. The global area under chickpea is 14.80 million ha, with production of 14.23 million tonnes and productivity of 962 kg/ha. In India, it is grown in an area of about 10.74 million ha with production of 9.88 million tonnes and productivity of 920 kg/ha. In Karnataka, it is grown in 0.95 m ha with production of 0.72 million tonnes and productivity of 757 kg/ha [2]. The reasons for low yield is due to incidence of diseases. The crop is known to be affected by number of soil-borne pathogens, some of which may be devastating. Chickpea suffers from about 172 pathogens consisting of fungi, bacteria, viruses and nematodes. Soil borne diseases such as wilt (*Fusarium oxysporum* f. sp. *ciceri*), dry root rot (*Macrophomina phaseolina*), black root rot (*Fusarium solani*), collar rot (*Sclerotium rolfsii*), and stem rot (*Sclerotinia sclerotiorum*) are important in reducing the yield of the crop. The foliar diseases *viz.*, Ascochyta blight (*Ascochyta rabiei*) and grey mould (*Botrytis cinerea*) are more severe in chickpea crop. The dry root rot (*M. phaseolina*) is a major constraint in the chickpea production as it is emerging as a potential threat to chickpea cultivation in semi-arid regions due to moisture stress and high temperatures during the flowering to pod filling stage [18]. The annual yield loss due to this disease alone is 10-20 per cent [24]. The dry root rot disease generally appears around flowering and podding time. The disease

may also appear at seedling stage, however, the susceptibility of the plant increases with age. The disease generally appears when day temperature is more than 30 $^0$C and soil moisture content of 60 per cent. Drooping of petioles and leaflets is confined to those at the very top of the plant. Sometimes when rest of the plant is dry, the top most leaves are chlorotic. The leaves and stems of affected plants are usually straw colored. The lower portion of the tap root usually remains in the soil when plants are uprooted. The tap root is dark and is devoid of most of its lateral and finer roots. Dark, minute sclerotial bodies can be seen on the roots or inside the wood [12]. It is necessary to conduct survey for dry root rot disease to get comprehensive information on disease distribution, level of severity, extent of spread and to locate hot spots of disease. The information on the management of disease is negligible. Hence detailed investigation was undertaken to evaluate the bio-agents and fungicides.

## MATERIALS AND METHODS

### Survey for dry root rot of chickpea in southern parts of Karnataka

An intensive roving survey on the incidence of dry root rot caused by *M. phaseolina* was conducted in chickpea growing areas of Karnataka *viz.,* Tumakuru, Chitradurga, Bengaluru rural, Bengaluru urban, Chikkaballapur, Chikkamagaluru, Hassan and Mysuru districts. In each district, minimum two taluks, from each taluk 2-9 villages and from each village minimum two chickpea fields were selected. During survey per cent disease incidence was recorded and infected plant samples were collected for further isolation of pathogen. The following formula was used to calculate disease incidence. Per cent Disease Incidence = (Number of disease plants/ Total number of plants) × 100

The antagonistic microorganisms *viz.*, *Trichoderma harzianum*, *T. viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated for their antagonistic effect in *in-vitro* conditions against *M. phaseolina* by dual culture technique. In dual culture technique, twenty ml of sterilized and cooled potato dextrose agar was poured into sterile petriplates. Fungal antagonists was evaluated by inoculating the pathogen at one side of petriplate and the antagonist inoculated at exactly opposite side of the same plate by leaving 3-4 cm gap. In case of bacterial antagonist evaluation, two mycelial discs of pathogen were inoculated and bacterial antagonist was streaked in the center of the plate. Each treatment was replicated four times. After required period of incubation *i.e.,* after control plate reached 90 mm diameter, the radial growth of pathogen was measured. Per cent inhibition over control was worked out according to equation [22].

### *In-vitro* evaluation of Fungicides

Different systemic fungicides *viz.* carbendazim, tebuconazole, thiophanate methyl, difenoconazole and propiconazole were evaluated at different concentrations of 50, 100, 250 and 500 ppm and contact fungicides *viz.,* mancozeb , chlorothalonil, captan and thiram were evaluated at different concentrations of 250, 500, 750 and 1000 ppm and combi-products fungicides *viz.,* tricyclozole 4 % + mancozeb 62 % WP, hexaconazole 4 % **+** zineb 68 % WP, carboxin 37.5 % + thiram 37.5 % WP and carbendazim 12 % + mancozeb 63 % WP were evaluated at different concentrations of 250, 500, 750 and 1000 ppm. The fungicides were tested against *M. phaseolina* by adopting 'Poisoned food technique'. The required concentrations of chemicals were prepared and incorporated into sterilized, cooled potato dextrose agar. Twenty ml of cooled medium was poured into petridishes and all plates were inoculated with actively growing five mm mycelia disc of pathogen. Three replications were maintained for each treatment. These plates were incubated at 27±1 °C for seven days, and colony diameter was recorded. Per cent inhibition of mycelial growth over control was calculated by using the formula of Vincent (1927) as follows: I= [(C-T)/C]x100, Where, I = Per cent inhibition of mycelium, C = Growth of mycelium in control, T = Growth of mycelium in treatment. Analysis and interpretation of the experimental data was done by employing completely randomised design (CRD) method for laboratory studies suggested by Panse and Sukathme (1985).

## RESULTS AND DISCUSSION

### Survey for dry root rot of chickpea

Among the different taluks surved chickpea dry root rot incidence varied from 18.61 per cent to 61.24 per cent. Among the districts the highest mean disease incidence was recorded in Chitradurga district (56.10 %) followed by Tumakuru (52.57 %), Bengaluru urban (51.13 %), Bengaluru rural (40.53 %), Chikkaballapur (36.41 %), Mysuru (32.09 %), Hassan (29.97 %) and Chikkamagaluru (24.73 %) (Table 1). This wide variation in disease incidence may be due to the change in the environment conditions, cultivars used, variation in date of sowing and cultural practices followed.

The highest DRR incidence (40.00 per cent) in village Shangus and lowest (4.11 per cent) in village Naina [7]. The high incidence of the disease in such field might be due to the fact that the disease perpetuates through debris in field. The same type of observation was recorded by the survey conducted during *rabi* season of 2008 which revealed that dry root rot of chickpea varied from locality to locality due to

different soil conditions (Black/Red soil conditions), cultivars used, cultivation practices and environmental conditions prevailing over these tracts. The higher incidence may be due to exposure of chickpea plants to moisture stress conditions evidenced during *rabi* season, which ultimately led to more production of sclerotia of *Rhizoctonia* sp. on chickpea plants roots [11, 20]. The disease incidence of dry root rot of soybean was significantly high when inoculated seedlings were water stressed and grown at low soil moisture level [26]. Similarly, conducted a survey in *rabi* cropping season in different chickpea growing locations of central (Madhya Pradesh and Maharashtra) and southern (Andhra Pradesh, Telangana and Karnataka) India [19]. The maximum dry root rot incidence was observed in Telangana (18.28 %) and the least in Maharashtra (5.38 %). Disease occurrence was observed irrespective of cropping system, soil types and cultivars. The disease incidence was low in the irrigated fields compared to rainfed fields. The variation in disease incidence was due to diversified weather conditions, variation in sowing dates, different crop growth stages.

***In-vitro* evaluation of bioagents against *M. phaseolina***

The results of the study indicated that all the antagonists significantly inhibited mycelial growth of *M. phaseolina* and per cent inhibition ranged from 62.96 to 81.44. Among fungal and bacterial antagonists, the maximum inhibition of mycelial growth was observed in *T. harzianum* (Th-55) isolate with 81.44 per cent followed by *B. subtilis* (75.56 %), *T. viride* (Tv-27) with 74.44 per cent, *P. fluorescens* (70.37 %), *T. harzianum* (IIHR) with 70.04 per cent, *T. viride* (B) with 64.44 per cent and minimum inhibition was observed in *T. harzianum* (GKVK) with 62.96 per cent (Table 2).

The suggested mechanisms for biocontrol of plant pathogens by *Trichoderma* were antibiosis, lysis, competition and mycoparasitism (Cook and Baker, 1983). *T. harzianum* and *T. viride* both suppressed the growth of *M. phaseolina* and this may be due to coiling and disintegration of hyphae of the test fungus resulting in the loss of competitive saprophytic ability [10, 1, 5, 6, 10 and 16]. Several scienstist were evaluated the antagonism of *T. viride, P. fluorescens* and *B. subtilis* isolates against *R. bataticola* (*M. phaseolina*) collected from pigeonpea, chickpea, green gram, cluster bean, field pea, cotton, okra and safflower in *in-vitro*. *T. viride* was most effective in the inhibition of the various isolates of *R. bataticola* (100 %), followed by *B. subtilis* (87.41 - 92.89 %) and *P. fluorescens* (73.98 - 78.94 %).

***In-vitro* evaluation of systemic fungicides against *M. phaseolina***

Among five systemic fungicides evaluated, tebuconazole, carbendazim, difenoconazole and propiconazole inhibited cent per cent mycelial growth at all concentrations. Whereas, the least inhibition of 53.33, 89.26 and 93.33 per cent was observed in case of thiophanate methyl at 50, 100 and 250 ppm concentration respectively. However, cent per cent inhibition was observed at 500 ppm concentration (Table 3).

***In-vitro* evaluation of contact and combi - products fungicides against *M. phaseolina***

Among the contact fungicides *viz.,* mancozeb, thiram, chlorothalonil and captan evaluated against *M. phaseolina,* mancozeb recorded cent per cent inhibition of mycelial growth at all the concentrations of given treatment followed by thiram which recorded the inhibition of mycelial growth of 65.56, 78.52, 85.19 and 91.11 per cent at 250, 500, 750 and 1000 ppm concentration respectively, followed by captan 48.15, 68.89, 77.41 and 84.44 per cent at 250, 500, 750 and 1000 ppm concentration respectively. However, chlorothalonil recorded the least inhibition of 46.30, 54.81, 68.15, 79.63 % mycelial growth at 250, 500, 750 and 1000 ppm concentration, respectively (Table 4).

Among the combi - products *viz.,* tricyclozole 4 % + mancozeb 62 % WP, hexaconazole 4 % **+** zineb 68 % WP, carboxin 37.5 % + thiram 37.5 % WP and carbendazim 12 % + mancozeb 63 % WP evaluated against the *M. phaseolina,* carboxin 37.5 % + thiram 37.5 % WP and carbendazim 12 % + mancozeb 63 % WP showed cent per cent inhibition of mycelia growth at all concentrations (250, 500, 750 and 1000 ppm) followed by tricyclozole 4 % + mancozeb 62 % WP with 79.63, 89.26, 100.00 and 100.00 per cent at 250, 500, 750 and 1000 ppm concentration respectively . The least inhibition was observed in hexaconazole 4 % **+** zineb 68 % WP with 62.22, 75.93, 82.59 and 97.41 per cent at 250, 500, 750 and 1000 ppm concentration respectively (Table 4). Similarly carbendazim being benzimidazole group of fungicide, it interferes with energy production and cell wall synthesis of fungi [12]. It might be due to carbendazim induced nuclear instability [4] by disturbing the mitosis and meiosis [8, 9, 14, 15, 17, 23 and 25].

## CONCLUSION

*M. phaseolina* is primarily seed and soil-borne fungal pathogen. In chickpea, infected seeds and microsclerotia surviving in the soil are the major source of primary inoculum. The pathogen also has wide host range. Since 75 per cent cultivation of chickpea in India is under rainfed, the crop faces severe moisture stress at flowering to podding stage which predisposes the crop to dry root rot development. Diseases with limited distribution are economically important locally because of continuous changes in cultural practices, human interventions and climate change. The improved practices for disease

management will pave a way to mitigate losses caused by dry root rot and improves livelihoods of the poor farmers.

**Table 1. Mean dry root rot disease incidence of chickpea in different taluks and districts of southern Karnataka**

| Sl.No. | District | Taluk | Mean disease incidence (Taluk) | Mean disease incidence (District) |
|---|---|---|---|---|
| 1. | Tumakuru | Tumakuru | 48.78 | 52.57 |
| | | Tiptur | 55.72 | |
| | | Pavagoda | 53.21 | |
| 2. | Chitradurga | Hiriyur | 53.29 | 56.10 |
| | | Hosadurga | 61.24 | |
| | | Challakere | 53.99 | |
| | | Chitradurga | 55.88 | |
| 3. | Bengaluru Rural | Devanahalli | 37.07 | 40.53 |
| | | Dodballapura | 45.71 | |
| | | Hoskote | 38.82 | |
| 4. | Bengaluru Urban | Anekal | 42.97 | 51.13 |
| | | Bengaluru south | 59.29 | |
| 5. | Chikkaballapur | Gauribidanur | 26.96 | 36.41 |
| | | Chikkabalpura | 45.85 | |
| 6. | Chikkamagaluru | Chikkamagaluru | 18.61 | 24.73 |
| | | Kadur | 28.79 | |
| | | Tarikere | 26.79 | |
| 7. | Hassan | Hassan | 27.90 | 29.97 |
| | | Arsikere | 40.21 | |
| | | Chanarayapatana | 21.81 | |
| 8. | Mysuru | K. R. Nagara | 34.92 | 32.09 |
| | | Hunsur | 29.26 | |

**Table 2. *In-vitro* evaluation of bioagents against *M. phaseolina* by dual culture technique**

| Sl. No. | Bio agents | Per cent inhibition over control* |
|---|---|---|
| 1. | *Trichoderma viride* (B) | 64.44 (53.40) |
| 2. | *Trichoderma viride* (Tv-27) | 74.44 (59.63) |
| 3. | *Trichoderma harzianum* (GKVK) | 62.96 (52.51) |
| 4. | *Trichoderma harzianum* (IIHR) | 70.04 (56.81) |
| 5. | *Trichoderma harzianum* (Th-55) | 81.44 (64.48) |
| 6. | *Pseudomonas fluorescens* | 70.37 (57.02) |
| 7. | *Bacillus subtilis* | 75.56 (60.37) |
| 8. | Control | 0.00 |
| S. Em ± | | 0.15 |
| CD at 1% | | 0.63 |

***** Figures in parentheses are arc sin angular transformed values

**Table 3. *In-vitro* evaluation of systemic fungicides against *M. phaseolina***

| Sl. No. | Fungicides | Per cent inhibition of mycelial growth* | | | | |
|---|---|---|---|---|---|---|
| | | Concentrations | | | | |
| | | 50 ppm | 100 ppm | 250 ppm | 500 ppm | Mean |
| 1. | Thiophanate methyl | 53.33 (46.91) | 89.26 (70.87) | 93.33 (75.03) | 100.00 (90.00) | 83.98 (66.41) |
| 2. | Tebuconozole | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) |

| | | | | | | |
|---|---|---|---|---|---|---|
| 3. | Carbendazim | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) |
| 4. | Difenoconazole | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) |
| 5. | Propiconazole | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) |

| S. Em ± | Fungicides | Concentration | Fungicide × concentration |
|---|---|---|---|
| | 0.06 | 0.05 | 0.11 |
| CD at 1% | 0.22 | 0.19 | 0.43 |

***** Figures in parentheses are arc sin angular transformed values

**Table 4. *In-vitro* evaluation of contact and combi- products fungicides against *M. phaseolina***

| Sl. No. | Fungicides | Per cent inhibition of mycelial growth* | | | | |
|---|---|---|---|---|---|---|
| | | Concentrations | | | | |
| | | 250 ppm | 500 ppm | 750 ppm | 1000 ppm | Mean |
| **Contact fungicides** | | | | | | |
| 1. | Mancozeb | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) |
| 2. | Thiram | 65.56 (54.07) | 78.52 (62.39) | 85.19 (67.37) | 91.11 (72.65) | 80.10 (63.50) |
| 3. | Chlorothalonil | 46.30 (42.88) | 54.81 (47.76) | 68.15 (55.64) | 79.63 (63.17) | 62.22 (52.07) |
| 4. | Captan | 48.15 (43.94) | 68.89 (56.10) | 77.41 (61.62) | 84.44 (66.77) | 69.72 (56.62) |
| **Combi-products** | | | | | | |
| 5. | Tricyclazole 4 %+ Mancozeb 62 % WP | 79.63 (63.17) | 89.26 (70.87) | 100.00 (90.00) | 100.00 (90.00) | 92.22 (73.81) |
| 6. | Hexaconazole 4 % **+** Zineb 68 % WP | 62.22 (52.07) | 75.93 60.62) | 82.59 (65.34) | 97.41 (80.74) | 79.54 (63.11) |
| 7. | Carboxin 37.5 %+ Thiram 37.5 % WP | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) |
| 8. | Carbendazim 12 % + Mancozeb 63 % WP | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) |

| S. Em ± | Fungicides | Concentration | Fungicides × concentration |
|---|---|---|---|
| | 0.11 | 0.08 | 0.22 |
| CD at 1 % | 0.41 | 0.29 | 0.82 |

***** Figures in parentheses are arc sin angular transformed

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**CITATION OF THIS ARTICLE**

Jyothi, V. and Muhammad Saifulla. In-*vitro* evaluation of Bioagents and chemical fungicides against dry root rot of chickpea caused by *Macrophomina phaseolina* in southern parts of Karnataka. Bull. Env. Pharmacol. Life Sci., Vol 6 Special issue 2, 2017: 412-417