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Evaluation of combined application of newer insecticides with some fungicides to control groundnut stem rot, *Sclerotium rolfsii sacc.* under *in vitro*

■ P.VENKATARAO* AND K. MANJULA¹

Department of Agricutural Entomology, Uttar Banga Krishi Viswavidyalaya, Cooch Behar, PUNDIBARI (W.B.) INDIA ¹Department of Agricutural Entomology, Sri Venkateswara Agricultural College (ANGRAU) TIRUPATI (A. P.) INDIA

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ABSTRACT

An *in-vitro* experiment was conducted to determine the effect of different insecticides, fungicides and their combination treatments on radial colony growth of *Sclerotium rolfsii* Sacc. by following poison food technique in PDA medium. Two contact (chlorpyriphos 0.05%, thiodicarb 0.075%), three stomach and contact poison insecticides (chlorfenapyr 0.002%, spinosad 0.0025 and emamectin benzoate 0.003%), among the fungicides one systemic (hexaconazole 0.25%), one contact (Mancozeb 0.25%) and their ten insecticide-fungicide combinations were evaluated with recommended doses against *S. rolfsii* in laboratory during the year 2009-2010 at S.V. Agricultural College, Tirupati. Chlorpyriphos 0.05 per cent (70.33%) was the best insecticide to restrict the fungal growth effectively followed by emamectin benzoate (34.03%). The two fungicides *i.e.* mancozeb 0.25 per cent, hexaconazole 0.2 per cent and their combinations with insecticides were found effective in reducing (cent per cent) the growth of *S. rolfsii*. Fungicides do not shown any antagonism when mixed with insecticides.

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INTRODUCTION

Email: venkatarao16@gmail.com

*Corresponding author:

Groundnut (*Arachis hypogaea* L.) is very important crop of developing countries which contributes around 95 per cent of world production (Nautiyal, 2002). In India it is considered to be an important crop as oilseed crop. The annual production of oil from it is estimated to be in 8 million tons (Patil, 2009). As groundnut is susceptible to wide range of micro-organisms which may include fungi, viruses, mycoplasma, nematodes and bacteria, it may have contributed in overall yield losses. *Sclerotium rolfsii* Sacc. (teleomorph, *Athelia rolfsii*) is among those fungal soil-borne root pathogens which causes severe stem-rot in groundnut plant. It is commonly occurs in the tropics, sub-tropics and other warm temperate regions of the world causing root rot, stem rot, wilt and foot rot on more than 500 plant species in about 100 families including almost all the agricultural and horticultural crops (Aycock, 1966). The losses due to stem rot in groundnut caused by *S. rolfsii* was reported to the tune of 27 per cent and reduction in pod number and weight to the extent of 23 and 38 per cent, respectively (Singh and Mathur, 1953). The loss of yield caused by pathogen infection generally is 25 per cent, but sometimes it reaches 80 - 90 per cent in some cases (Grichar and Bosweel, 1987).

Sclerotium rolfsii Sacc. is a soil borne polyphagous fungal pathogen is characterized by white fluffy, branched, septate mycelium and spherical or irregular shaped brown sclerotia, which range from 0.5-2.0 mm in diameter and at maturity, resemble mustard seed. The mycelium of *S. rolfsii* survives best in sandy soils, whereas, the sclerotia survive best in moist, aerobic conditions found at the soil surface. Management of the pathogen is quite difficult due to its polyphagus nature, off-seasonal survivality and wide adaptability. Present investigation was made to screen out the effective insecticides, fungicides and their combinations for developing suitable management strategy by integration of both insecticides, fungicides and their combinations against the pathogen.

On the basis of above information in the present investigation, five insecticides and two fungicides and their combination treatments were evaluated for their efficacy against Sclerotium rolfsii in vitro. The information on the compatibility of new pesticides is very much scanty. A chemical possessing both insecticidal and fungicidal properties, if identified, would help in reducing the pesticide consumption and pesticide load in the environment. Combined application of newer insecticides and commonly used fungicides may result either in synergism / antagonism between the two. However, in view of the complexities arising from the use of chemical pesticides would help in reducing the pesticide consumption and pesticide load in the environment. Hence, there is every need to generate information on effective and economical combinations; so as to advise the farmers about detrimental effect of combinations which would result in wasteful expenditure as well as time and crop loss.

MATERIAL AND METHODS

General laboratory procedures :

Glassware cleaning :

For all laboratory experimental studies, lorning and borosil glassware's were used. The glassware's were boiled for half an hour and then washed with detergent powder followed by cleaning in tap water and then rinsed into distilled water.

Sterilization:

All glassware used in the studies were sterilized in autoclave at 1.1 kg/cm² pressure for 21 minutes and then dried in hot air oven at 55°C. Potato dextrose agar media used in the experiments was sterilized at 1.1 kg/cm² pressure for 15 minutes.

Isolation and maintenance of the pathogen :

S. rolfsii can be isolated from different plant parts *viz.*,collar region of the affected portion of the plant tissue (Yella Goud, 2011). Stem (Kajal Kumar and Sen, 2000). Potato Dextrose Agar (PDA) was found to be the best supporting medium for *S. rolfsii* (Naidu, 2000).

Peeled potatoes	: 200 g
Agar	: 20 g
Dextrose	: 20 g
Distilled water	: 1000 ml

pH was adjusted to 6.8 by using 0.1 N HCl and 0.1 N NaOH. Medium was sterilized at $121.6^{\circ}C$ (37.5 kg pressure 6.25 cm⁻²) for 15 minutes in an autoclave.

The fungicidal action of the insecticides and fungicides and their combination treatments on the growth of the fungus were assessed by poisoned food technique (Table 1).

Poisoned food technique:

Fungicidal action of selected insecticides and fungicides was studied *in-vitro* by poison food technique (Zentmeyer,1955) was essentially followed. The test fungus was allowed to grow on poisoned potato dextrose agar medium and the colony diameter was recorded on per cent inhibition basis over control. The different insecticides and fungicides (emulsifiable concentrates, soluble concentrates, wettable powders and soluble granules) to be tested were quantified with mono- pan electrical balance or graduated micro pipettes and added to the medium after cooling to obtain a particular

concentration of the product. Each chemical was tested at recommended concentrations. Requisite quantities of each insecticide and fungicide were accurately added in to 250 ml conical flask containing molten agar separately. Care was taken to make up the volume of medium with chemicals to 100 ml. To each flask two mg of streptomycin powder was added to prevent bacterial growth. The contents were well stirred and mixed thoroughly and poured on to three Petridishes (90 mm diameter) equally at 20 ml per Petridish. Discs of 0.5 cm were cut with a sterilized cork borer from the outer margins of the seven day old culture grown on agar media was used as inoculum and transferred aseptically in to the centre of each Petridish in an inverted position so that the fungus would be in direct contact with the poisoned nutrient medium. The Petridishes were kept in the incubator at 30°C along with checks were kept on PDA without toxicant. Each treatment was replicated thrice. The diameter of the radial growth of colonies in each of the treatments was measured in four directions lengthwise and breadth wise and mean was calculated. The observations were made from 72 hours after inoculation and diametric growth was recorded daily and were compared with the check to evaluate for their fungi toxic properties.

The selected insecticides and their combinations were tested for their compatibility with the fungicides and the fungicides alone generally recommended for soil drenching *viz.*, mancozeb (0.25%), hexaconazole (0.2%) and insecticides *viz.*, chlorpyriphos (0.05%), spinosad (0.002%), chlorfenapyr (0.002%), thiodicarb (0.075%) and emamectin benzoate (0.003%) were tested against pathogen *S. rolfsii.*

Radial growth :

The radial growth of the fungus was measured using a measuring scale at three days after inoculation of test fungus and compared with control.

$$\mathbf{R} = \frac{\mathbf{C} - \mathbf{T}}{\mathbf{C}} \mathbf{x} \mathbf{100}$$

where,

- R = Per cent reduction of radial growth of the test fungus.
- C = Radial growth of test fungus in untreated control (mm)
- T = Radial growth of test fungus in treatment (mm).

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Pathogenicity tests :

Artificial inoculation of the plants with the pathogen was done by different methods. Soil inoculation by the pathogen was studied by several workers, Dange (2006) and Datur and Bindu (1974). Seedling root dip inoculum was used to induce sclerotial wilt in bell pepper (Chowdary, 1997). Plants were artificially inoculated by spreading 20 seeds (0.6 g) of sorghum based inoculums around the stems at 6-8 leaf stage modified from (Block *et al.*, 2007). A non- inoculated treatment is also included as control. The inoculum is placed beneath the soil surface in close proximity of the stems of the plants. The water was supplied regularly to avoid stress.

Stastical analysis :

The data obtained in this experiments were statistically analyzed by using Completely Randomized Design (CRD). The data pertaining to percentages were angularly transformed (Table A). Results were analyzed by following appropriate statistical methods as per the procedure suggested by Panes and Sukhatme (1978).

RESULTS AND DISCUSSION

Certain chemicals developed as insecticides are known to exhibit fungistatic or fungitoxic properties. Therefore *in vitro* evaluation of insecticides regarding their fungicidal properties was made, against *S.rolfsii*. In the present study five insecticides *viz.*, chlorpyriphos 20 EC, spinosad 45 SC, chloefenapyr 10 SC, thiodicarb 75 WP and emamectin benzoate 5 SG and two fungicides *viz.*, mancozeb 75 WP and hexaconazole 5 EC were tested at recommended concentrations. *In vitro* evaluation of insecticides mixed with fungicides revealed that cent per cent mycelial growth was inhibited. It was interesting to note that chlorpyriphos was exhibited fungitoxic property in the study by inhibiting the fungal growth.

Hence, from the present findings (Table 1). It is clear that both the fungicides were significantly effective at their recommended doses in inhibiting the mycelial growth of the pathogen, *S. rolfsii* as compared to control was observed. Among the treatments the mean radial growth of *S. rolfsii* varied from 0.0 to 90 mm (Fig.1-8). From the results, it is clear that the radial growth of *S. rolfsii* was adversely affected by the fungicides. The effective fungicides, probably may act as antifungal agent and impacts its poisoning effect on metabolic process of pathogen, therefore, the growth of the pathogen might be adversely affected. These results were supported by many workers, Arunasri et al. (2011), who reported that the Triazoles (Hexaconazole, Propiconazole, Difenconazole) were highly inhibitive to the growth of S. rolfsii. The results obtained are in correlation with Johnson et al. (2008), who reported the inhibition of S. rolfsii pathogen with hexaconazole and propiconazole at 0.1 per cent and 0.2 per cent. Radhaiah (2012) also reported that mancozeb 0.2 per cent completely suppressed the pathogen. Deepthi (2014) also reported that thiophenate methyl, hexaconazole, propiconazole were effective against suppressing the pathogen, S. rolfsii at both 0.1 per cent and 0.2 per cent concentrations under In vitro. Both the fungicides i.e mancozeb and hexaconazole exhibited cent per cent inhibition of growth of the fungus S. rolfsii. The present results are similar with the observations of Narayana and Srivastava (2003) where mancozeb and hexaconazole completely inhibited growth of S. rolfsii at three concentrations tested i.e. 250, 500 and 1000 ppm. Such inhibition was also

observed by Ramdoss and Siva Prakasam (1987b) in case of carbendazim which produced the largest inhibition zone (50.64 mm) of *Macrophomina phaseolina*.

The present results are also in accordance with the work of Johnson and Subramanyam (2000) who observed complete inhibition in radial growth of S. rolfsii with hexaconazole and propiconazole. Similar results were also reported by Isaiah et al. (2005). Rakholiya (2010) reported that cent per cent growth inhibition of S. rolfsii was found in propiconazole (0.025%) and mancozeb (0.20%). Further, Harlapur (1988) noticed the complete inhibition of mycelial growth of S. rolfsii by agallol and Diathane M-45. Propiconazole was found highly effective in inhibiting the mycelial growth of S. rolfsii. Bindu Madhavi and Bhattipolu (2011) reported that, in vitro evaluation of nine fungicides by poison food technique showed that tebuconazole and combination of carbendazim+mancozeb were effective in inhibiting the mycelial growth (94.1%) followed by difenconazole (93.3%).

Regarding the combining effect, both the fungicides

Tr. No.	Particulars	Mean radial growth (mm)*	Per cent reduction of growth over control	Per cent growth over control
T_1	Chlorpyriphos 20EC @ 0.05%	26.66 ^d	70.33 (57.02) ^a	29.67 (33.02) ^d
T_2	Spinosad 45 SC @ 0.002%	64.66 ^b	28.10 (31.96) ^c	71.90 (57.99) ^a
T ₃	Chlorfenapyr 10 SC @ 0.002%	64.00 ^b	28.83 (32.43) °	71.17 (57.54) ^a
T_4	Thiodicarb 75 WP @ 0.075%	62.66 ^b	30.33 (33.40) [°]	69.67 (56.60) ^b
T ₅	Emamectin benzoate 5% SG@ 0.003%	59.34 °	34.03 (35.67) ^b	65.97 (54.27) ^c
T ₆	Mancozeb 75 WP @ 0.25%	0.00 ^e	0.00 (0.00) ^d	0.00 (0.00) ^e
T ₇	Hexaconazole 5 EC @ 0.2%	0.00 ^e	0.00 (0.00) ^d	0.00 (0.00) ^e
T_8	Chlorpyriphos 20 EC @ 0.05%+ mancozeb 75 WP @ 0.25%	0.00 ^e	0.00 (0.00) ^d	0.00 (0.00) ^e
T ₉	Chlorpyriphos 20 EC @ 0.05%+ hexaconazole 5 EC @ 0.2%	0.00 ^e	0.00 (0.00) ^d	0.00 (0.00) ^e
T ₁₀	Spinosad 45 SC @ 0.002% + mancozeb 75 WP @ 0.25%	0.00 ^e	0.00 (0.00) ^d	0.00 (0.00) ^e
T ₁₁	Spinosad 45 SC @ 0.002% +hexaconazole 5 EC @ 0.2%	0.00 ^e	0.00 (0.00) ^d	0.00 (0.00) ^e
T ₁₂	Chlorfenapyr 10 SC@ 0.002% + mancozeb 75WP @ 0.25%	0.00 ^e	0.00 (0.00) ^d	0.00 (0.00) ^e
T ₁₃	Chlorfenapyr 10 SC @ 0.002%+ hexaconazole 5 EC @ 0.2%	0.00 ^e	0.00 (0.00) ^d	0.00 (0.00) ^e
T ₁₄	Thiodicarb 75 WP @ 0.075% + mancozeb 75WP@ 0.25%	0.00 ^e	0.00 (0.00) ^d	0.00 (0.00) ^e
T ₁₅	Thiodicarb 75 WP @ 0.075%+ hexaconazole 5 EC @ 0.2%	0.00 ^e	0.00 (0.00) ^d	0.00 (0.00) ^e
T ₁₆	Emamectin benzoate 5%SG@ 0.003%+ mancozeb 75 WP @ 0.25%	0.00 ^e	0.00 (0.00) ^d	0.00 (0.00) ^e
T ₁₇	Emamectin benzoate 5% SG@ 0.003%+ hexaconazole 5 EC @ 0.2%	0.00 ^e	0.00 (0.00) ^d	0.00 (0.00) ^e
T ₁₈	Control	90.00 ^a	-	-
S.E. ±		0.53	0.74	0.60
C.D. (F	P = 0.05)	1.53	2.13	1.73

Figures in parenthesis indicates square root transformed ($\sqrt{X + 0.5}$) values *= Average of 3 replications

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Photographs: In Vitro compatibility of newer insecticides with certain fungicides in the control of stem rot, S.rolfsii Sacc.

Plate1: Pure culture of Sclerotium rolfsii

Plate2: Per cent inhibition of growth of *Sclerotium rolfsii* in the medium treated with mancozeb



- Plate3: Per cent inhibition of growth of *Sclerotium rolfsii* in the medium treated with hexaconazole
- Plate 4: Per cent inhibition of growth of *Sclerotium rolfsii* in the medium treated with chlorpyriphos, chlorpyriphos+ mancozeb and chlorpyriphos+ hexaconazole

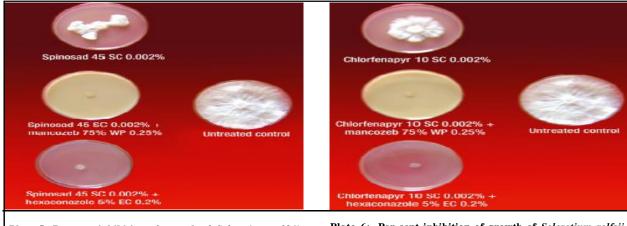
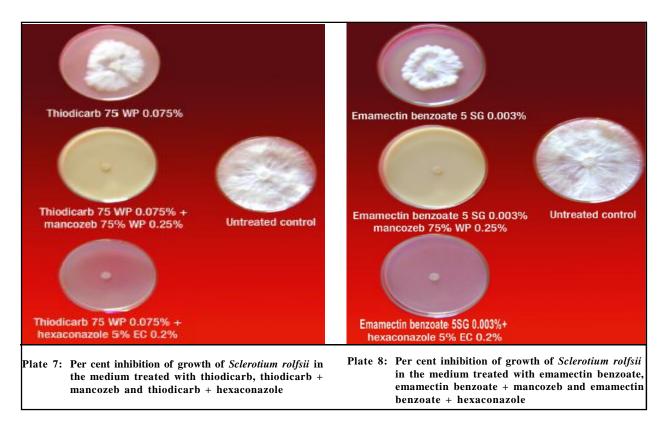


Plate 5: Per cent inhibition of growth of *Sclerotium rolfsii* in the medium treated with spinosad, spinosad + mancozeb and spinosad + hexaconazole Plate 6: Per cent inhibition of growth of *Sclerotium rolfsii* in the medium treated with chlorfenapyr, chlorfenapyr + mancozeb and chlorfenapyr + hexaconazole

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at recommended concentrations were tested in combination with five insecticides. Amongst all the tested pesticide treatments both the fungicides and their combinations with insecticides proved to be the most effective against S. rolfsii in which cent per cent (100%) inhibition in radial growth was recorded in a recommended concentrations (Table 1). There is no inhibition of fungicidal activity of the fungicides by the insecticides when mixed. Although, no specific literature is available in respect of bio-efficacy of the above chemical combinations against S. rolfsii for comparison, it is evident from the available literature on related insecticides of selected groups that mancozeb and hexaconazole is biologically and chemically compatible in vitro with insecticides. Thus, additive effect of combination of these insecticides with fungicides accounted for increased mycelial inhibition over fungicide alone. Regarding the bio-efficacy of mancozeb and hexaconazole in combination with insecticides, as evident from the mycelial inhibition the efficacy of mancozeb and hexaconazole increased with all the five insecticides tested. The fungicidal toxicity of such mixtures was reported by Ramdoss and Siva Prakasam (1987) in case of carbosulfan+carbendazim on fungus, Macrophomina

phaseolina which produced 56.06 mm inhibition zone. These findings were further supported by the findings of Khan *et al.*(1989) who recorded significant enhancement of fungicidal activity against *Drechslera oryzae* with the combination products of captafol+ monocrotophos. These results were also in agreement with the findings of Shukla and Lal (1989) who observed effective control of powdery mildew of field pea with the combination product of carbendazim + monocrotophos.

As evident from the mycelial inhibition, chlorpyriphos showed greater efficacy out of five insecticides tested. Minimum radial growth (26.66 mm) of fungus, *S.rolfsii* was observed in chlorpyriphos 0.05 per cent among insecticide treatments alone (Table 1). The next best treatment was emamectin benzoate 0.003 per cent with radial growth of 59.37mm. Maximum radial growth 64.66 mm, 64.00 mm and 62.66 mm were recorded in spinosad 0.002 per cent, chlorfenapyr 0.002 per cent and thiodicarb 0.075 per cent, respectively among insecticidal treatments. The data further revealed that per cent inhibition growth over control was less in other insecticides except chlorpyriphos (0.05%) because of lack of fungicidal property. Only chlorpyriphos was found to be fungistatic in action some extent. These findings are in agreement with those of Johnson et al. (2008) who reported that chlorpyriphos completely inhibited the pathogen growth (S. rolfsii) at one step lower (1500 ppm) and at recommended concentration (2000 ppm). According to Kucharek and Edmondson (1991) also, chlorpyriphos at 500 ppm suppressed the growth of S. rolfsii due to vapours emitted by the organophosphorus insecticide (chlorpyriphos) were inhibitory to the growth of S. rolfsii. Backman and Hammpmd (1981) published his results that the active ingradient and inert ingradients of emulsifiable formulation of Lorsban 4 EC showed synergism. There by suppressing the stem rot fungus, S. rolfsii. The findings of Dharamvir et al. (1973) were also in support of the present study as they revealed that the vapours emitted by the organophosphorus insecticide were inhibitory to the growth of Helminthosporium oryzae, Apsergillus niger, Colletotrichum gloeosporiodes and Rhizoctonia bataticola in varying proportions.

Maximum per cent reduction mycelial growth was observed in the organophosphorus insecticide chlorpyriphos (0.05%) was found to be superior in inhibiting (70.33% inhibition) the radial growth of the pathogen *S. rolfsii* among the insecticides (Plates 1-8). Significant difference was observed in per cent inhibition of growth of the pathogen with regard to all insecticides tested. Spinosad (0.002%), chlorfenapyr (0.002%), thiodicarb (0.075%) and emamectin benzoate (0.003%) were inhibited the per cent growth inhibition of 28.10, 28.83, 30.33 and 34.03 per cent, respectively.

Minimum per cent growth was observed in the organophosphorus insecticide chlorpyriphos (0.05%) was found to be superior (29.67% inhibition) the radial growth over control among the insecticides (Plates 1-8). Significant difference was observed in per cent growth of the pathogen with regard to all insecticides tested. Spinosad (0.002%), chlorfenapyr (0.002%), thiodicarb (0.075%) and emamectin benzoate (0.003%) were observed the per cent growth over control of 71.90, 71.17, 69.67 and 65.97 per cent, respectively.

Conclusion :

In the present study efficacy of commonly used fungicides, mancozeb, hexaconazole tested against the soil born fungal pathogen, *S. rolfsii* tested under *in vitro* both the fungicides completely inhibited the growth of the mycelium. The combining effect of fungicides with insecticides indicated that mancozeb and hexaconazole were compatible with chlorpyriphos 0.005 per cent, spinosad 0.002 per cent, chlorfenapyr 0.002 per cent, thiodicarb 0.075 per cent and emamectin benzoate 0.003 per cent. The data revealed that both the fungicides and its combinations with insecticides were completely inhibited the growth of *S. rolfsii*. Among insecticides, chlorpyriphos 0.02 per cent is the best insecticide to restrict the fungal growth effectively followed by emamectin benzoate.

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