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In vitro evaluation of antifungal agents against green muscardine fungi, *Metarhizium Anisopliae* and *Nomuraea Rileyi*

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Abstract: *In vitro* study of green muscardine fungi with fungicides and botanicals at their recommended doses was studied. Among the fungicides, carbendazim (0.025 and 0.1%) and formalin (0.4 and 0.8%) inhibited the complete growth of *Metarhizium anisopliae* and *Nomuraea rileyi* on seventh day of evaluation. The botanicals were found ineffective against the both the fungi. However, eucalyptus leaf powder 10 per cent (39.76%) and turmeric powder (28.26%) inhibited the maximum growth of *Metarhizium anisopliae* and *Nomuraea rileyi,* respectively.

Key words: Antifungal, Botanicals, Fungicides, Green muscardine

Introduction

The major hurdle in commercial mulberry silkworm rearing is disease infection *viz.,* pebrine, grasserie, flacherie and muscardine caused by protozoa, virus, bacteria and fungus. Among the disease, muscardine caused by the fungal pathogen is most contiguous and common inflicting significant loss in cocoon yield. Nearly a dozen species of fungi cause infection and are identified by the colour and appearance of dead silkworms. The white and green muscardine are common during rainy and winter and the loss in cocoon crop ranged from 30 to 40 per cent (Chandrasekaran and Nataraju, 2013). The source of infection is mainly due to mummified cadaver, contaminated leaves and rearing appliances (Nirupama, 2014). In recent years, the entomopathogens *viz., Metarhizium anisopliae* and *Nomuraea rilyei* are being widely used in the management of many crop pests. Increased use of entomopathogens has increased the pathogens load in the environment and the chances of cross infection to silkworms are very high. In commercial silkworm rearing, the fungal infection is prevented by dusting lime powder or dithane M-45 at 0.1 to 0.2 per cent as bed disinfectant. Many new broad spectrum fungicides available in the market were not tried for muscardine disease management. Similarly many botanicals are known to posses antifungal properties and not much work has been done on their efficacy in fungal disease management.

Material and methods

Pure culture of *Metarhizium anisopliae* and *Nomuraea rileyi* obtained from Institute of Organic Farming, University of Agricultural Sciences, Dharwad was multiplied by using Saborauds Maltose Agar Media (SMAY). The cultured plates were incubated at 28^0 C for 7 to 10 days. The fungal spores from SMAY plated were mixed with distilled water and 0.02 per cent Tween-80 to produce the spore suspension. The conidial load was determined with a Neubauer haemocytometer. Finally, through serial dilution method spore suspensions containing $1x10^8$ conidia/ml was obtained. *In vitro* study was conducted by using poison food technique (Nene and Thapliyal, 1997). Culture of *Metarhizium anisopliae* and *Nomuraea rileyi* were grown on SMAY media for seven days at 28°C temperature for growth inhibition studies. Potato Dextrose Agar (PDA) medium was used with requisite quantity of fungicides and botanicals to get desired concentration as mentioned. Required quantities of individual antifungal agents were added separately into sterilized molten and cooled potato dextrose agar so as to get the desired concentration of fungicides and botanicals. Later, 20 ml of the poisoned medium was poured into sterilized petri plates. Seven days old fungus culture of small disc (0.8cm) was cut with sterile cork borer and transferred aseptically. One such disc was placed at the centre of each agar plate. Control treatment was maintained without adding any fungicide and botanicals to the medium. Two replications were maintained for each treatment. Later, the plates were incubated at room temperature and radial growth was measured when fungus attained maximum growth in the control plates. The data collected was analyzed in completely randomized design statistical method.

Results and discussion

All the five fungicides inhibited the growth of *M. anisopliae* fungus significantly as compared to control. Among the fungicides, carbendazim (0.025 and 0.1%), chlorothalonil (0.1 and 0.3%) and formalin (0.4 and 0.8%) found highly detrimental to the fungus by inhibiting cent per cent fungal growth on seventh day after inoculation, followed by dithane M-45 0.3 per cent (78.31%) and 0.1 per cent (73.49%) and propiconazole 0.1 per cent (55.02%) and 0.025 per cent (48.59%) (Table1). The fungicides used against the fungus were having different mode of action and the fungicides having systemic mode of action found to be most effective as compared to contact fungicides. The different mode of action of the fungicides might be the reason for varying degrees of fungus growth inhibition. The results of the present study are in agreement with Rachappa *et al.* (2007) as they observed carbendazim, propiconazloe, chlorothalonil and hexaconazole

Table 1. *In vitro* evaluation of fungicides against green muscardine, *Metarhizium anisopliae*

Fungicides	Conc. (%)	Colony growth (mm)					
		3 DAI	Inhibition (%)	5 DAI	Inhibition (%)	7 DAI	Inhibition (%)
Chlorothalonil	0.1	0.00	100.00(89.96)*	0.00	100.00(89.96)	0.00	100.00(89.96)
Chlorothalonil	0.3	0.00	100.00(89.96)	0.00	100.00(89.96)	0.00	100.00(89.96)
Propiconazole	0.025	14.67	33.33(35.25)	17.67	39.08(38.68)	21.33	48.59(44.17)
Propiconazole	0.1	11.33	48.48(44.11)	15.00	48.28(44.00)	18.67	55.02(47.86)
Carbendazim	0.025	0.00	100.00(89.96)	0.00	100.00(89.96)	0.00	100.00(89.96)
Carbendazim	0.1	0.00	100.00(89.96)	0.00	100.00(89.96)	0.00	100.00(89.96)
Dithane M-45	0.1	7.33	66.67(54.72)	9.00	68.97(56.13)	11.00	73.49(58.99)
Dithane M-45	0.3	6.00	72.73(58.50)	8.67	74.11(60.84)	9.00	78.31(62.22)
Formalin	0.4	0.00	100.00(89.96)	0.00	100.00(89.96)	0.00	100.00(89.96)
Formalin	0.8	0.00	100.00(89.96)	0.00	100.00(89.96)	0.00	100.00(89.96)
Control	-	22.00	0.00(0.00)	29.00	0.00(0.00)	41.50	0.00(0.00)
S.Em±	-	0.22	1.01	0.26	0.88	0.34	0.82
C.D. (1%)	-	0.64	2.91	0.74	2.55	0.98	2.35

*Figures in parentheses are arcsine transformed values
DAI: Days after inoculation

Table 2. *In vitro* evaluation of botanicals against green muscardine, *Metarhizium anisopliae*

Botanicals	Conc. (%)	Colony growth (mm)					
		3 DAI	Inhibition (%)	5 DAI	Inhibition (%)	7 DAI	Inhibition (%)
Eucalyptus oil	5	20.33	7.58(15.97)*	26.33	9.20(17.65)	37.67	9.24(17.69)
Eucalyptus oil	10	16.67	24.24(29.48)	24.00	17.24(24.52)	34.33	17.27(24.55)
Acorus calamus	5	20.33	7.58(15.97)	24.33	16.09(23.64)	36.67	11.65(19.95)
Acorus calamus	10	14.67	33.33(35.25)	22.00	24.14(29.42)	33.00	20.48(26.90)
Curcuma longa powder	5	20.00	9.09(17.54)	26.33	9.20(17.65)	40.33	2.81(9.65)
Curcuma longa powder	10	18.00	18.18(25.23)	24.67	14.94(22.73)	38.00	8.43(16.87)
Eucalyptus globules leaf powder	5	18.67	15.15(22.90)	20.00	31.03(33.84)	31.33	24.50(29.66)
Eucalyptus globules leaf powder	10	15.00	31.82(34.33)	17.33	40.23(39.35)	25.00	39.76(39.08)
Control	-	22.00	0.00(0.00)	29.00	0.00(0.00)	41.50	0.00(0.00)
S.Em.±	-	0.38	1.75	0.35	1.22	0.32	0.78
C.D. (1%)	-	1.10	4.98	1.01	3.48	0.92	2.21

*Figures in parentheses are arcsine transformed values
DAI: Days after inoculation

Table 3. *In vitro* evaluation of fungicides against green muscardine, *Nomuraea rileyi*

Sl. No.	Fungicides	Conc. (%)	Colony growth (mm)				
			3 DAI	Inhibition (%)	5 DAI	Inhibition (%)	7 DAI
Inhibition (%)							
Chlorothalonil	0.1	7.67	69.93(56.72)*	10.67	67.35(55.13)	14.33	69.93(56.72)
Chlorothalonil	0.3	6.33	75.16(60.08)	8.67	73.47(58.97)	12.67	73.43(58.95)
Propiconazole	0.025	10.33	59.48(50.44)	11.67	64.29(53.28)	14.33	69.93(56.72)
Propiconazole	0.1	8.67	66.01(54.32)	9.67	70.41(57.02)	11.33	76.23(60.80)
Carbendazim	0.025	0.00	100.00(89.96)	0.00	100.00(89.96)	0.00	100.00(89.96)
Carbendazim	0.1	0.00	100.00(89.96)	0.00	100.00(89.96)	0.00	100.00(89.96)
Dithane M-45	0.1	0.00	100.00(89.96)	0.00	100.00(89.96)	0.00	100.00(89.96)
Dithane M-45	0.3	0.00	100.00(89.96)	0.00	100.00(89.96)	0.00	100.00(89.96)
Formalin	0.4	0.00	100.00 (89.96)	0.00	100.00(89.96)	0.00	100.00(89.96)
Formalin	0.8	0.00	100.00(89.96)	0.00	100.00(89.96)	0.00	100.00(89.96)
Control	-	25.50	0.00(0.00)	32.67	0.00(0.00)	47.67	0.00(0.00)
S.Em.±	-	0.24	0.94	0.18	0.56	0.18	0.38
C.D. (1%)	-	0.69	2.71	0.52	1.60	0.52	1.10

*Figures in parentheses are arcsine transformed values
DAI: Days after inoculation

were found highly detrimental to *M. anisopliae.* Kotwal *et al.* (2012) observed the higher inhibition by carbendazim at recommended (70.48%) and double dose concentration (84.77%), followed by thiram. In the present study, among the botanicals, eucalyptus leaf powder 10 per cent and 5 per cent (39.76 and 24.50%) inhibited maximum growth of the fungus. They were followed by *Acorus calamus* 10 per cent (20.48%), eucalyptus oil 10 per cent (17.27%), *Acorus calamus* 5 per cent (11.65%), eucalyptus oil 5 per cent (9.24%), turmeric powder 10 per cent (8.43%) and 5 per cent (2.81%) (Table 2). Chavan *et al.* (2011) and Isaiarasu *et al.* (2011) reported inhibitory effect of botanicals against *Beauveria bassiana* in *in vitro* evaluation.

In vitro evaluation of fungicides against *Nomuraea rileyi* growth resulted in cent per cent inhibition by carbendazim (0.025 and 0.1%), dithane M-45 (0.1 and 0.3%) and formalin (0.4 and 0.8%) and found highly detrimental to the fungus. They were followed by chlorothalonil at 0.3 per cent (73.43%) and 0.1 per cent (69.93%) and propiconazole at 0.025 per cent (69.93%) and 0.1 per cent (76.23%) (Table 3). The present results are in conformity with the earlier work of Patil *et al.* (2013) as they observed that carbendazim and dithane M-45were found highly detrimental to *Nomuraea rileyi*. The botanical extracts found to be less effective in inhibiting the fungal growth. Eucalyptus oil 5 and 10 per cent showed 7.70 and 13.29 per cent inhibition. *Acorus calamus* 5 and 10 per cent showed 11.89 and 15.39 per cent inhibition. Turmeric powder 5 and 10 per cent inhibited 11.89 and 28.26 per cent fungal growth, respectively (Table 4). Eucalyptus leaf powder 5 and 10 per cent recorded 7.00 and 22.38 per cent inhibition (Table 4). With respect to other botanicals, Patil *et al.* (2013) reported 50 to 55 per cent inhibition after seven days of inoculation with *Annona squamosa* seed extract (5%), *Polyantha longifolia* (5%) and *Parthenium hysterophorous* (10%). This variation in the results obtained with botanicals may be due to differences in plant species used and presence of antifungal bio ingredients at varying levels.

Table 4. *In vitro* evaluation of botanicals against green muscardine, *Nomuraea rileyi*

Botanicals	Conc. (%)	Colony growth (mm)					
		3 DAI	Inhibition (%)	5 DAI	Inhibition (%)	7 DAI	Inhibition (%)
Eucalyptus oil	5	23.33	8.50(16.94)*	28.67	12.25(20.48)	44.00	7.70(16.10)
Eucalyptus oil	10	20.00	21.57(27.66)	25.33	22.46(28.28)	41.33	13.29(21.37)
Acorus calamus	5	22.00	13.73(21.74)	27.33	16.34(23.83)	42.00	11.89(20.16)
Acorus calamus	10	18.33	28.10(32.00)	24.33	25.52(30.33)	40.33	15.39(23.09)
Curcuma longa powder	5	20.67	18.95(25.80)	26.67	18.38(25.38)	42.00	11.89(20.16)
Curcuma longa powder	10	19.33	24.18(29.44)	20.67	36.74(37.30)	24.67	28.26(32.10)
Eucalyptus globules leaf powder	5	21.33	16.34(23.83)	28.00	14.29(22.20)	44.33	7.00(15.34)
Eucalyptus globules leaf powder	10	18.67	26.80(31.16)	25.67	21.44(27.57)	37.00	22.38(28.22)
Control	-	25.50	0.00(0.00)	32.67	0.00(0.00)	47.67	0.00(0.00)
S.Em±	-	0.26	1.01	0.27	0.83	0.48	1.01
C.D. (1%)	-	0.73	2.88	0.77	2.37	1.36	2.86

*Figures in parentheses are arcsine transformed values

DAI: Days after inoculation

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