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EFFECTIVENESS OF FUNGICIDES, METHANOLIC BOTANICAL EXTRACTS AND ANTAGONISTS AGAINST INVASIVE *Phytophthora palmivora* INCITING LEAF SPOTS OF CHINESE FAN PALM

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ABSTRACT

Effectiveness of fungicides, methanolic botanical extracts and antagonists against invasive *P. palmivora* inciting leaf spots of Chinese fan Palm was carried out. Best fungicide in restraining the development of the organism, in plunging request were Range, Difenconazole, Acrobat MZ, Precure and Thiophenate methyle as they caused 100, 100, 97, 92 and 91 percent decrease over the control in mycelial development of *P. palmivora*. While, Propmeb, Sulfur and Mancozeb were slightest powerful in hindering the mycelial development of parasite as they caused 41, 35 and 33 percent lessening in mycelial development. Best methanolic plant extricates in restraining the development of the organism, in plummeting request were Moringa Leaf Concentrate, Neem Leaf Concentrate, Akk Leaf Concentrate, Garlic Concentrate and Datura Leaf Concentrate as they caused 87, 86, 76, 76 and 70 percent diminishment over the control in mycelial development *P. palmivora*, separately. There was continuous trend of reduction in mycellial growth with increase in fungicide and methanollic plant extract concentration. All the antagonistic microorganisms reduced the mycelial growth of *P. palmivora* significantly. *Pseudomonas fluorescence* has produced largest inhibition zone (5.5 mm) followed by *Penicillium* spp. which has produced (5 mm) inhibition zone. *Basillus fortis* was the third most effective antagonist against *P. palmivora* with 4.5 mm inhibition zone. *Aspergilus nigar* proved to be least effective antagonistic microorganisms which has produced 2 mm inhibition zone as compared to other antagonists.

Keywords: Chinese fan Palm, *Phytophthora palmivora,* fungicides, botanicals and antagonists, inhibition zone.

INTRODUCTION

Chinese fan palm (*Livistona chinesis)* belongs to the family *Arecaceae* [1]. It is also known as fountain palm. Roughly 28 species of *Arcaceae* family are known. As Chinese fan palms are tolerant to drought so they can be grown in wide range of soils. Medium shade or even full sunlight supports their growth. At the top of Chinese fan palm there is single unbranched stem on which green fan shaped leaves appear. These leaves give fountain like appearance inspiring the cognomen; Chinese fountain

palm. Its leaves are 3 to 4 feet across and nearly 6 feet long. The sheath of leaves is fibrous and brown in colour. When fruits become ripened they change into blue-black colour [2]. Young palm trees have spiny Petioles, but adult palm trees have no spines. Petioles have length of 2.5 meters and they are flat on ventral surface and convex on dorsal surface. Juvenile specimens are slow to form trunk. Apart from the lower portion where the surface is wrinkled and spongy, remaining trunk is overlaid with bases of the leaves. Forty to sixty leaves are being organized at the top of trunk to form crown.

This species is fit for growing indoors and it can also be used in Stour parking lot islands. It can be used in lawns. One can also use this plant close to deck and near residential tree. They can be grown in narrow soil spaces. When they are grown at distance of 10 feet in street they form close canopy. It is usually grow as ornamental plant [3].

Seed of *L. chinensis* is used to treat various types of cancers. The products of Chinese fan palm seeds, additionally called as Pukuizi in Chinese, utilized as a society antitumor operator in China for the treatment of gastric growth, nasopharyngeal carcinoma, chorionic carcinoma, leukemia and different ailments [4]. *L. chinensis* has antioxidants, so it has the ability to cure cancers because of its nature to bind, denature or fixing the damage which result in the action of active oxygen species [5]. This herb is also anticipated to get excellent natural antioxidants that have ability to avoid cancers.

L. chinensis is attacked by several pathogens including bacteria and many fungal species. One of the serious fungal diseases of Chinese palms is *Ganoderma* butt rot. Diamond scale disease, which is also very important among its diseases caused by *Phaeochoropsis neowashingtoniae.* This disease is produce water soaked lesions that later turn black and become large and turn into shiny, diamond shaped fruiting bodies. These fruiting bodies can be seen on both the upper and lower surfaces of leaf blades and petioles. Diamond scale turns leaves to yellow and brown then become dead prematurely reducing crown of leaves and gives an unattractive appearance of landscape subject.

Beside of these diseases leaf spot or leaf blight which is caused by *P. palmivora* is also a very important disease of Chinese fan palm. It destroys the leaves of plant and reducing the ornamental value of Chinese fan palm for which it is grown. This research was carried out to study the effectiveness of fungicides, methanolic botanical extracts and antagonists to find a cheap and safe management of *P. palmivora* inciting leaf spot or leaf blight of Chinese fan palm.

MATERIALS AND METHODS

The sensitivity of mycelial growth of *P. palmivora* against 7 different fungicides: Mancozeb (80%), Thiophanate Methyl (70%), Propmeb (70%), Benomyl (50%), Panconazol (100%), Difenoconazole (25%) and Sulphur (80%) at concentrations viz. 0.1, 0.5, 1, 5, 10, 20, 50, 100, 200 and 500 mg/L and methanolic plant extracts: Garlic Extract, Akk Leaf Extract, Neem Leaf Extract, Moringa Leaf Extract and Datura Leaf Extract were evaluated at different concentrations that are i.e. 1, 2, 5, 10, 15 ml/100ml by using modified Borum and Sinclair's technique [6].

Each concentration of fungicides and methanolic botanical extracts was filled in as a sole treatment. A deliberate amount of each of the fungicide/methanolic botanical

extract was altered to autoclaved malt agar medium for getting required concentrations. Malt extract agar medium without fungicide/methanolic botanical extract served as control. Fifteen milliliter of altered and non-altered medium was poured in each 90mm diameter petri dish. After solidification 5mm agar plugs containing *P. palmivora* mycelium were cut from 10 days old culture and placed in the center of each Petri dish with the help of sterilized needle. These vaccinated petri plates were brooded at 25 ±2ºC. Information on outspread mycelial development of *P. palmivora* in centimeters was recorded at an interim of 24 hours till the mycelia developed to full on the control and information were dissected measurably to see the distinctions among different fungicides and methanolic botanical extracts.

Seven antagonistic micro-organisms *viz; Aspergilus snigar*, *Bacillus subtilus*, *Penicillium spp., Pseudomonas fluorescence, Bacillus fortis*, *Trichoderma harzianum* and *Trichoderma viride* were used to evaluate suitable antagonistic organism against the pathogen *P. palmivora.* Both the antagonistic micro-organism and the pathogen were inoculated side by side at the opposite ends of the petri plates having 20 ml malt extract agar medium. Three Petri dishes were used for each antagonist and the same number was kept as control. In control the pathogen was alone plated on either side of the plate at the periphery. Inoculated petri dishes were brooded at 25 ±2ºC for 7-10 days. Inhibition zones produced between the antagonistic micro-organisms and *P. palmivora* was measured to evaluate their effectiveness.

RESULTS AND DISCUSSION

Fungitoxic effect of seven fungicides viz Mancozeb, Propeneb, Benomyl, Thiophenate methyle, Panconazole, Difenconazole and Sulphur at nine concentrations 1, 5, 10, 50, 100, 150 200, 300 and 500 ppm were tested *in vitro* with fungicide amended agar medium. Investigation of change demonstrated exceedingly noteworthy effects of fungicides, their focuses and the association amongst fungicides and their fixation for reducing the mycelial development of the *P. palmivora*. The outcomes got on the fungitoxicity of fungicides against *P. palmivora in vitro* are exhibited in (Table 1). Effectiveness of fungicides changed extraordinarily among every single tried fungicide and their fixations. As a general pattern, there was a critical reduction in mycelial development of the parasite with rise in fungicide concentration in all the tried fungicides over the control. In any case, when development of the parasite because of different fungicide focuses following seven days of hatching at 25 ± 2°C looked at based on examination of methods for all fungicide concentrations, Spectrum and Difenconazole turned out to be the best as it had given the greatest control (100 percent) on every one of the concentrations tried. While Sulfur ended up being the minimum compelling (69 percent) at most astounding fixation (500 ppm) tried. Tumbler and Precure had repressed the mycelial development of *P. palmivora* totally at low convergence of 5 and 10 ppm, separately. Benomyl had additionally repressed the mycelial development of *P. palmivora* totally at three convergences of 200, 300 and 500 ppm and Popeneb repressed the development totally at 500 ppm. None of the other tried fungicides have totally checked the mycelial development of *P. palmivora*. Along these lines, the best fungicides in restraining the development of the parasite, in sliding request were Spectrum, Difenconazole, Acrobat, Precure and Thiophenate methyle as they caused 100,

100, 97, 92 and 91 percent lessening over the control in mycelial development *P. palmivora*, separately. While, Propmeb, and Sulfur and Mancozeb were slightest viable in hindering the mycelial development of parasite as they caused 41, 35 and 33 percent diminishment in mycelial development over the control, separately (Table 1).

In-vitro evaluation of various methanolic Botanical Extracts by poisoned agar technique against mycellial growth of *Phytophthora palmivora*

Fungitoxic efficacy of five methanolic botanical extracts *viz;* Garlic Extract, Akk Leaf Extract, Neem Leaf Extract, Moringa Leaf Extract and Datura Leaf Extract at five concentrations *viz*; 1, 2, 5, 10 and 15 ml/100ml were applied through botanical extract amended agar medium. Examination of change demonstrated exceedingly noteworthy outcomes among methanolic plant removes, their focus and association between botanical extricates and their concentrations. Analysis of difference indicated profoundly critical outcomes among methanolic botanical extracts, their concentrations and interaction between botanical extracts and their concentrations.

The outcomes got on the fungitoxicity of methanolic botanical extracts against *P. palmivora in vitro* are presented in (Table 2). Viability of every one of the six methanolic organic concentrates shifted extraordinarily among each other and their concentrations. All in all, there was a diminishing in mycellial development *P. palmivora* with an expansion in convergence of methanolic botanical extracts. Be that as it may, when development of the pathogen in light of different methanolic botanical extracts focuses after a hatching time of seven days at 25 ± 2°C was analyzed, Moringa leaf extract turned out to be the best as it had given the greatest control (85 percent) trailed by Neem leaf remove (73 percent) on 2 ml/100ml concentration. While Datura leaf extract was proved to be the least effective (82 percent) at highest concentration (5 ml/100ml) (Table 2). Hence, the best methanolic plant extracts in restraining the development of the parasite, in descending order was Moringa Leaf Extract, Neem Leaf Extract, Akk Leaf Extract, Garlic Extract and Datura Leaf Extract as they caused 87, 86, 76, 76 and 70 percent reduction over the control in mycelial development *P. palmivora*, individually (Table 2). It is concluded that there was an overall trend of reduction in mycelial growth of *P. palmivora* with an increase in the concentration of methanollic plant extracts.

In-vitro evaluation of antagonistic microorganisms against mycelial growth of *Phytophthora palmivora*

Seven antagonistic micro-organisms *viz; Aspergilus snigar*, *Basillus subtilus*, *Penicillium spp., Pseudomonas fluorescence, Basillus fortis*, *Trichoderma harzianum* and *Trichoderma viride* were tested *in vitro* for their antagonistic effect on *P. palmivora*. Analysis of variance showed highly significant effect of antagonistic microorganisms on the mycellial growth of *P. palmivora* by culturing them on opposite sides of the petri plate (Table 3). All the antagonistic microorganisms had positive effect on the growth of the test fungus and have created inhibition zones of various sizes. All the antagonistic microorganisms reduced the growth of *P. palmivora* significantly as compared to control (Fig. 3). *Pseudomonas fluorescence* has produced largest inhibition zone (5.5 mm) followed by *Penicillium spp.* which has produced (5 mm) inhibition zone as compared to other

Table 1. Percent inhibition of mycelial growth of *Phytophthora palmivora*

	1 ppm	5 ppm	10 ppm	50 ppm	100 ppm	150 ppm	200 ppm	300 ppm	500 ppm
Spectrum	100	100	100	100	100	100	100	100	100
Difenconazole 25 EC	100	100	100	100	100	100	100	100	100
Benomyl	73	77	84	83	85	94	100	100	100
Mancozeb 80 wp	18	10	33	21	11	24	38	48	96
Thiophanate Methyl	81	88	88	89	98	96	92	89	97
Propeneb 70wp	7	10	18	13	20	31	77	91	100
Sulfer	16	18	21	27	17	38	48	59	69
Penconazole	69	51	83	86	91	92	88	97	97
Precure	53	75	100	100	100	100	100	100	100
Acrobat	76	100	100	100	100	100	100	100	100

CV = 8.53

Table 2. Percent inhibition of mycelial growth of *Phytophthora palmivora*

Treatment	1 ppm	2 ppm	5 ppm	10 ppm	15 ppm
Garlic	22	58	100	100	100
AKK	34	63	85	100	100
Neem	55	73	100	100	100
Datura	24	43	82	100	100
Moringa	51	85	100	100	100

CV = 4.14

antagonistic organisms. *Basillus fortis* was the third most effective antagonist against *P. palmivora* with 4.5 mm inhibition zone. *Aspergilus snigar* proved to be least effective antagonistic microorganisms which has produced 2 mm inhibition zone as compared to other antagonists.

Preliminary assessment of the relative impact of seven fungicides and three homeo-fungicides on the mycelial development of *P. palmivora* uncovered that the viability of fungicides in hindering the mycelial development of the pathogen varied a great deal. Subsequently, the best fungicides in repressing the development of the organism, in plummeting request were Spectrum (Difenoconazole + Propiconazole), Difenconazole 25 EC, Acrobat, Precure and Thiophenate methyle as they caused 100, 100, 97, 92 and 91 percent decrease over the control in mycelial development *P. palmivora*, respectively. While, Propmeb, and Sulfur and Mancozeb were minimum viable in restraining the mycelial development of organism as they caused 41, 35 and 33 percent decrease in mycelial development over the control, individually.

Hislop, E. C. [7] used different fungicides to control *P. palmivora*. His study revealed that in *in vitro* conditions phenyl mercury nitrate at the rate of 0.02 ppm and fentin acetate at the rate of 0.2 ppm showed very serious effect on the growth of four isolates of *P. palmivora* when grow on cassava agar. Moreover when these chemical mixed with captan, meneb and dithiocarbamate-copper were also showed very good results against *P. palmivora*. In field conditions the both Bordeaux mixture and carbide Bordeaux gave good results on the other side the captan mixture was completely ineffective. The application of 0-15% fentin acetate at weekly basis reduced the damage cause by *P. palmivora* at significant level.

Table 3. *In vitro* effects of different antagonistic organisms on growth inhibition of *Phytophthora palmivora*

Treatment	Inhibition zones (mm)
Aspergilus nigar	2
Trichoderma harzianum	4
Trichoderma viride	3.5
Penicillium spp.	5
Basillus subtitus	4
Basillus fortis	4.5
Pseudomonas fluorescens	5.5
Control	0

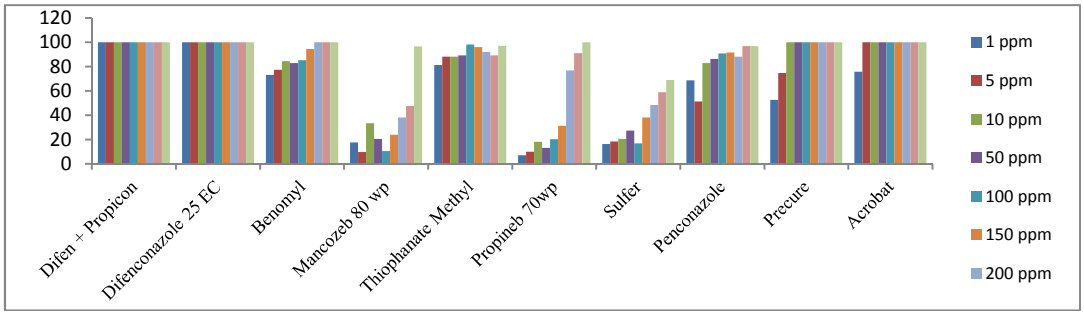

Fig. 1. Percent inhibition of mycelial growth of *Phytophthora palmivora*

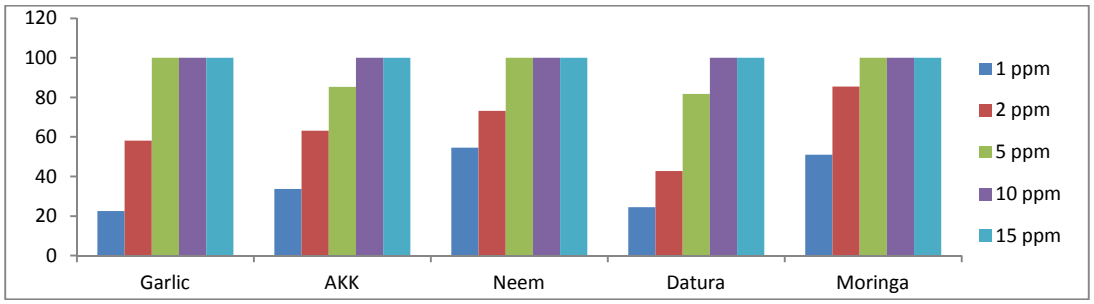

Fig. 2. Percent inhibition of mycelial growth of *Phytophthora palmivora*

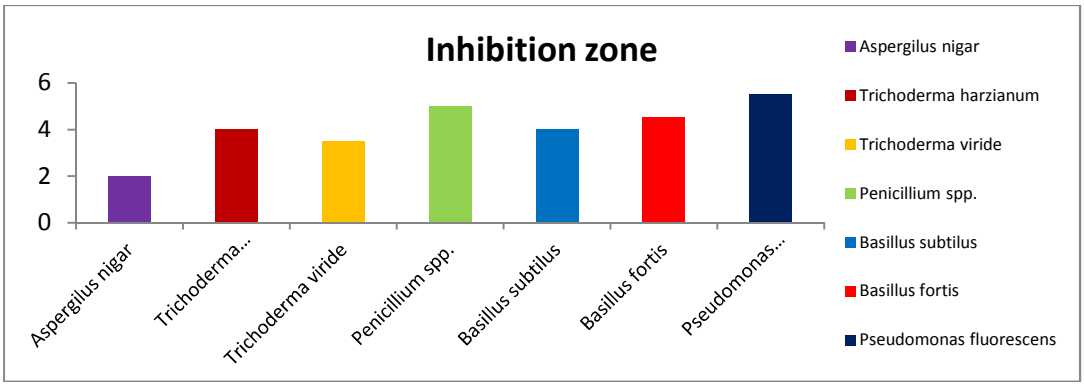

Fig. 3. Effect of different antagonistic organisms on growth inhibition of *Phytophthora palmivora*

Ho [8] controlled the *P. palmivora causing* Phytophthora fruit rot of papaya by using preventive fungicide like mancozeb and basic copper sulfate. Holderness [9] controlled *P. palmivora* by foliar spray and trunk injection of potassium phosphonate and metalaxyl/cuprous oxide sprays on cocoa plants in field trail. In this experiment it was seen that foliar spray give poor results as compare than either metalaxyl/cuprous oxide spray or trunk injection. Perpetual and broad utilization of these manufactured pesticides are posturing significant issue to the life supporting frameworks because of their leftover harmfulness [10]. Considering the malicious impacts of manufactured pesticides on life supporting frameworks, there is a critical need to scan for elective methodologies for the administration of plant pathogens. Strains of a few bacterial animal varieties, for example, *Bacillus, Pseudomonas* and as of late the Rhizobium aggregate were found to viably control different soil-borne plant pathogens development under field and nursery conditions [11].

There was an overall trend of reduction in mycellial growth of *P. palmivora* with an increase in the concentration of methanollic plant extracts. Along these lines, the best methanolic botanical extracts in restraining the development of the organism, in descending order were Moringa Leaf Extract, Neem Leaf Extract, Akk Leaf Extract, Garlic Extract and Datura Leaf

Extract as they caused 87, 86, 76, 76 and 70 percent diminishment over the control in mycelial development *P. palmivora*, respectively.

Mpika et al. [12] studied 135 isolates of *Trichoderma* from cocoa plant and checked their antagonistic effect on *P. palmivora* using in vitro conditions. These identified isolates were including 64 as *T. virens*, 60 as *T. harzianum*, 7 as *T. spirale*, and two as *T. asperellum*. Out of these isolates 25 isolates restricted the mycelium growth of *P. palmivora* upto 50 percent out of which *T. virens* showed the best result. It reduced the mycelium growth of *P. palmivora* upto 98 percent. Hanada et al. [13] studied the antagonistic effect of *Trichoderma martiale* on *P. palmivora* in the presence of fungicide such as copper hydroxide and they studied that it had no effect on conidial germination of this *T. martiale* strain. The clear cut results of their study were that *T. martiale* had very high potential to control the *P. palmivora* even in the presence of fungicide. Timmusk et al. [14] studied the antagonistic effect of *Rhizobacterium paenibacillus Polymyxa* on oomycete plant pathogens *Phytophthora palmivora* and their results showed that *Paenibacillus polymyxa* reduce the colonization of *P. palmivora* by significant level.

All the antagonistic microorganisms reduced the growth of *Phytophthora palmivora* significantly but *Pseudomonas fluorescence* produced longer inhibition zone as compared to other antagonistic microorganisms and among the others *Penicillium spp., Basillus fortis, Basillus subtilus, Trichoderma harzianum, Trichoderma viride,* and *Aspergilus nigar* inhibited the growth of *P. palmivora* in descending order. *Pseudomonas fluorescence* has produced largest inhibition zone (5.5 mm) followed by *Penicillium spp.* which has produced (5 mm) inhibition zone as compared to other antagonistic organisms. *Basillus fortis* was the third most effective antagonist against *P. palmivora* with 4.5 mm inhibition zone. *Aspergilus snigar* proved to be least effective antagonistic microorganisms which has produced 2 mm inhibition zone as compared to other antagonists.

Awuah, [15] used *Ocimum gratissimum* (clove basil) and *Cymbopogon citratus* (lemon grass) as plants extract to control the *P. palmivora.* He compared these both extracts and the fungicide Kocide 101. In this experiment he found that clove basil extract was most effective against *P. palmivora* that suppress the mucillium growth of *P. palmivora* by 75% as compare to Kocide 101 by 2%. The lemon grass extract was also ineffective. Sporangia of *P. palmivora* had lost their infectiveness within an hour after treated with clove basil extract.

CONCLUSION

Effectiveness of Fungicides, methanolic plant extracts and antagonistic micro-organisms was carried out against invasive *P. palmivora* inciting leaf spots of Chinese fan Palm. As a general pattern, there was a significant decline in mycelial development of the growth with an expansion in fungicide fixation in all the tried fungicides over the control. So fungicides and plant extracts must be used in combination or alternatively to minimize the chances of fungicide resistance development in the pathogen.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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