Botanical Based Protection could be a Sustainable Alternative in Management of Castor (Ricinus communis) Wilt Caused by Fusarium oxysporum f.sp. ricini

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Abstract

Castor (Ricinus communis L.) experienced serious declines in yield caused by wilt pathogen Fusarium oxysporum f.sp. ricini. To control the wilt pathogen, commonly fungicides are used, which cause undesirable toxic effect on the environment. To minimize the pollution impact, there is an urgent need to develop alternative ecofriendly strategies. Therefore, to fill these knowledge gaps and to investigate potential botanical’s efficacy on the wilt pathogen F. oxysporum f.sp. ricini, an in vitro bioassay was conducted. Twenty-six botanicals were extracted at two different concentrations viz. 5 percent and 10 percent and were evaluated against the castor wilt pathogen, using poisoned food technique in terms of percent inhibition. The botanical henna showed significant (93.88 percent) inhibition of pathogen at 10 percent concentration, followed by neem cake (89.90 percent), ashoka (88.05 percent) and aloe (87.68 percent). Whereas at 5 percent concentration, botanical neem cake showed maximum inhibition (88.42 percent) followed by aloe (85.46 percent) and henna (83.70 percent). The plant extracts neem cake, aloe and henna significantly inhibited the pathogen growth at both the concentrations, indicating botanicals as potential future bio-fungicides. However, still further research needed to better understand the mechanisms underlining pathogen inhibition by plant extracts. Hence, the current study put forth that botanical based protection could be a potential alternative for the sustainable management of F. oxysporum f.sp. ricini.

Keywords
Castor, Fusarium oxysporum f.sp. ricini, Botanicals, Inhibition

Introduction

Castor (Ricinus communis L.) is an important non-edible oilseed crop. India ranks first in both area (10.96 lakh ha) and production (11.43 lakh tonnes) of castor in the world (Indiastat, 2013). In India Gujarat, Rajasthan and erstwhile Andhra Pradesh are the major castor producing states. However, in the past decade, the castor has been severely affected by wilt pathogen causing yield loss up to 85 percent depending on fungal inoculum level and environmental conditions (Dange, 2003). The castor wilt causal agent F. oxysporum f.sp. ricini is both soil and seed borne pathogen, colonize xylem vessels of host plant, and causing immense yield loses. In erstwhile Andhra Pradesh, wilt incidence
ranged from 5-60 percent, causing yield reduction of 1.86 kg/ha with each percent incidence of wilt disease (Chattopadhyay, 2000). Generally, fungicides are used against phytopathogenic fungi to control the plant diseases. But the continuous use of chemical fungicides in the management of plant disease impose harmful side effects on environment and has become a major threat to mankind. Hence, in recent years there has been increased awareness on toxic hazards of chemicals to crops, consumer and environment due to residual phytotoxicity and pollution effect. Developing eco-friendly and economical based protection to control plant diseases could be another best source (Yerukala et al., 2017; Yerukala et al., 2018). Include screening of plant products for their effective antifungal activity against the plant pathogens, could be another alternative to minimize the fungicide usage. Many studies have already been documented showing the botanicals fungitoxic nature and ability to inhibit the phytopathogen growth.

Studies include leaf extracts of neem and chinaberry inhibited wilt disease of tomato caused by F. oxysporum f.sp. lycopersici (Hassanein et al., 2008), others such as botanicals datura and isabgul inhibited F. oxysporum f.sp. cumini growth (Bhatnagar et al., 2004). Plant extracts of garlic, turmeric and black pepper reduced F. udum and F. oxysporum f.sp. ciceri growth (Shukla and Dwivedi, 2012). Additionally, floral extracts of Lantana camera inhibited spore germination and germ tube growth of Alternaria solani in vitro (Sundriyal, 1997). Despite many studies, there exits some research gap with respect to the plant extracts effect against F. oxysporum f.sp. ricini. Therefore, in present investigation, different plant species were evaluated for the possible presence of fungi toxic nature and efficacy against the mycelial growth of F. oxysporum f.sp. ricini in vitro. We aimed to address the following issues: 1) Find out the best botanical effective against the F. oxysporum f.sp. ricini among tested plant species for management of castor wilt 2) Plant part of the botanical that could be used effective against wilt pathogen growth reduction. 3. Suggestion or identification of botanicals, that could be economical for the farmers to manage the castor wilt disease.

Materials and Methods

All the experiments were carried out at Department of Plant Pathology, College of Agriculture, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, India. Twenty-six botanicals (Table 1, Fig. 2) were procured and their extracts were prepared by methodology mentioned by Mahapatra and Das, 2013. Efficacy of these plant extracts (Fig 3) against F. oxysporum f.sp. ricini (Fig. 1.) was evaluated under in vitro condition by using poisoned food technique (Kumar and Devendra, 2013). Percent inhibition over control was calculated using the formula mentioned below (Shalini et al., 2014).

\[ R = \frac{(X - Y)}{X} \times 100 \]

Where, \( R \) = Per cent growth reduction of test pathogen,

\[ X = \text{Radial growth of test pathogen in control (mm)} \]

\[ Y = \text{Radial growth of test pathogen in treatment (mm)} \]

The data obtained was transformed using angular transformations (Panse and Sukhatme, 1978) and was statistically analyzed using CRD (Completely Randomized Design), as per procedures suggested by Snedecor and Cochran, 1967). Both actual percentage values and their corresponding transformed values have been presented in Table 1.
Results and Discussion

All the plant species studied showed inhibition of *F. oxysporum* f.sp. *ricini* growth at different magnitude and the results are presented in Table 1 and figure 4, 5 and 6. At 5 percent concentration, neem cake showed maximum inhibition (88.42 percent). Other plant extracts at 5 percent which showed percent inhibition above 80 were aloe (85.46 percent), henna (83.70 percent), black pepper (82.96 percent) and tulsi (80.83 percent) (Fig. 4). At 10 percent concentration, henna was found superior with 93.88 percent inhibition. Other plant extracts at 10 percent which showed significant inhibition were neem cake (89.90 percent), ashoka (88.05 percent), aloe (87.68 percent), tulsi (85.83 percent), eucalyptus bark (85.74 percent), neem bark (85.55 percent), black pepper (83.79 percent), mint (82.31 percent), pongamia (81.57 percent) and calotropis leaf (81.01 percent) (Fig. 5). In the present study, all the plant extracts showed considerable growth reduction of *F. oxysporum* f.sp. *ricini* compared to control, however neem cake at both the concentrations performed better over other botanicals tested, and furthermore other best two botanicals include henna and aloe, while least inhibition of pathogen growth was recorded by lime. The results are in accordance with Joseph *et al.* 2008 who found that, neem extract effectively inhibited *F. solani* f.sp. *melongena* growth at 5, 10, 15 and 20 percent *in vitro*. Similarly, Chavan and Hegde, 2009 reported that neem seed kernel extract reduced the *F. solani* growth by 74.86 percent. The superiority of neem extract on inhibition of fungal pathogen was also mentioned by Sharma *et al.* 2011 on *F. oxysporum* f.sp. *lycopersici* in tomato. Similar results on neem effects were noted by Asit *et al.*, 2010 on *Alternaria* blight in malabar nut; *Alternaria alternata* in *Vicia faba* (Kumar *et al.*, 2005); on *F. pallidoroseum* and *F. oxysporum* (Gupta *et al.*, 1996). The fungitoxic property of neem could mainly attributed by the presence of various compounds includes azadirachtin, meliantriol, nimbin, nimbidin, 3-deacetylsalannin, salannol, salannin, 1,3 diacetylvilasinin, diacetylvilasinin, nimbandiol, azadirone, azadiradion, gedunin, nimbinene, nimocinolide, isonomocinolide, and isonomibocinolide, and trisulfides and tetrasulfides etc. (Koul *et al.*, 1990; Biswas *et al.*, 2002). Especially neem cake contains salannin, nimbin, azadirachtin and azadiradione as the major components (Del Serrone and Nicoletti, 2013) these phytochemicals could be possible niche for inhibition of the wilt pathogen growth (*F. oxysporum* f.sp. *ricini*). In addition, neem cake is a waste byproduct of neem oil extraction processes, used mainly as organic manure (Bureau of Indian Standards, Specification No. 8558). Moreover, neem cake, due to its lower cost in market and availability, farmers could procure easily and sustainably manage the wilt disease in castor.

In the study, apart from plant extract neem cake, henna and aloe also significantly inhibited wilt pathogen growth at both the concentration, suggesting its potentiality of botanical based eco-fungicide nature to manage the castor wilt. The fungitoxic nature of henna leaves against wilt pathogen could be possibly due to the presence of different chemical compounds such as hennatannic acid, lawson, 2-hydroxy-1:4 napthaquinone, lawson, gallic acid, glucose, mannotol, fats, resin, mucilage, other phytochemicals such as luteolin-7-o-glycoside, luteolin-3’-o-glycoside, stigmasterol, cosmosiiin (acacetin-7-o-glucoside), acacetin, p-coumaric acid, fraxetin, scopoletin, esculetin, 1,2-dihydroxy-4-o-glucosyloxy napthalene, lawsoniaside, lalioside, 2-methoxy-3-methyl-1, 4-napthaquinone, apiin, apigenin, lupeol, betulin and betulinic acid (Chaudhary *et al.*, 2010). In addition, botanical aloe also showed significant reduction of *F. oxysporum*
f.sp. *ricini* growth on par with henna, the fungi toxic nature of aloe could be due to the phytochemical compounds presence, include aloin, barbaloin, 10-(1',5'-anhydroglucosyl)-aloe-emodin-9-anthrone, aloe-emodin (Shelton, 1991).

Table 1 *In vitro* efficacy of different plant extracts against *F. oxysporum f.sp. ricini*

| Botanicals          | Botanical name                        | Family              | Plant part used | *Radial growth of *F. oxysporum* f.sp. *ricini* (mm) | *Per cent inhibition over control | *Radial growth of *F. oxysporum* f.sp. *ricini* (mm) | *Per cent inhibition over control |
|---------------------|---------------------------------------|---------------------|-----------------|-----------------------------------------------------|----------------------------------|-----------------------------------------------------|----------------------------------|
|                     |                                       |                     |                 | 5%                                                 |                                   | 10%                                                 |                                   |
| Garlic              | *Allium sativum* L                    | Liliaceae           | Clove           | 34.75                                               | 61.38 (51.56)                    | 31.91                                               | 64.53 (53.45)                    |
| Neem                | *Azadirachta indica* A                | Meliaceae           | Leaf            | 25.16                                               | 72.03 (58.10)                    | 19.91                                               | 77.87 (61.94)                    |
| Onion               | *Allium cepa* L                      | Liliaceae           | Bulb            | 34.08                                               | 62.12 (52.00)                    | 31.91                                               | 64.53 (53.45)                    |
| Ginger              | *Zingiber officinale*                 | Zingiberaceae       | Rhizome         | 48.66                                               | 45.92 (42.64)                    | 45.50                                               | 49.44 (44.66)                    |
| Tulsi               | * Ocimum sanctum* L                   | Labiataeae          | Leaf            | 17.25                                               | 80.83 (64.01)                    | 12.75                                               | 85.83 (67.86)                    |
| Pongamia            | *Pongamia pinnata* L                  | Fabaceae            | Leaf            | 24.25                                               | 73.05 (58.71)                    | 16.58                                               | 81.57 (64.55)                    |
| Custard Apple       | *Annona squamosa*                     | Annonaceae          | Leaf            | 52.66                                               | 41.48 (40.06)                    | 30.75                                               | 65.83 (54.21)                    |
| Lime                | *Citrus sinensis*                     | Rutaceae            | Leaf            | 69.66                                               | 22.59 (28.34)                    | 45.83                                               | 49.07 (44.45)                    |
| Mint weed           | *Lantana camara*                      | Verbenaceae         | Leaf            | 41.16                                               | 54.25 (47.58)                    | 34.83                                               | 61.29 (52.35)                    |
| Chili               | *Capsicum annum*                      | Solanaceae          | Fruit (pod)     | 50.50                                               | 43.88 (41.44)                    | 36.66                                               | 59.25 (51.35)                    |
| Ashoka              | *Polyalthia longifolia*               | Fabaceae            | Leaf            | 20.75                                               | 76.94 (61.31)                    | 10.75                                               | 88.05 (69.78)                    |
| Lantana             | *Hiptis suaveolens*                   | Lamiaceae           | Leaf            | 27.50                                               | 69.44 (56.43)                    | 24.91                                               | 72.31 (58.30)                    |
| Mint                | *Mentha Arvensis*                     | Lamiaceae           | Leaf            | 29.75                                               | 66.94 (54.92)                    | 15.91                                               | 82.31 (65.38)                    |
| Parthenium          | *Parthenium hysterophorus*            | Asteraceae          | Leaf            | 26.41                                               | 70.64 (57.22)                    | 20.00                                               | 77.77 (62.21)                    |
| Calotrope           | *Calotropis gigantean*                | Apocynaceae         | Leaf            | 26.50                                               | 70.55 (57.11)                    | 17.08                                               | 81.01 (64.46)                    |
| Calotrope           | *Calotropis gigantean*                | Apocynaceae         | Flower          | 22.83                                               | 74.62 (59.75)                    | 21.00                                               | 76.66 (61.53)                    |
| Tuja                | *Thuja occidentalis*                  | Cupressaceae        | Leaf            | 25.66                                               | 71.48 (57.70)                    | 23.08                                               | 74.35 (59.70)                    |
| Lantana bark        | *Lantana camara*                      | Verbenaceae         | Stem            | 25.91                                               | 71.20 (57.55)                    | 25.75                                               | 71.38 (57.64)                    |
| Neem bark           | *Azadirachta indica* A                | Meliaceae           | Bark            | 22.16                                               | 75.37 (60.35)                    | 13.00                                               | 85.55 (68.45)                    |
| Eucalyptus bark     | *Eucalyptus citridora*                | Myrtaceae           | Bark            | 21.16                                               | 76.48 (61.31)                    | 12.83                                               | 83.74 (67.82)                    |
| Ashoka bark         | *Polyalthia longifolia*               | Annonaceae          | Bark            | 25.66                                               | 71.48 (57.70)                    | 23.83                                               | 73.51 (59.01)                    |
| Henna tree          | *Lawsonia inermis*                    | Lythraceae          | Leaf Powder     | 14.66                                               | 83.70 (66.17)                    | 5.50                                                 | 93.88 (75.67)                    |
| Turmeric            | *Curcuma longa*                       | Zingiberaceae       | Powder          | 28.75                                               | 68.05 (55.56)                    | 21.91                                               | 75.64 (60.43)                    |
| Aloe vera           | *Alo vera*                            | Liliaceae           | Leaf            | 13.08                                               | 85.46 (67.57)                    | 11.08                                               | 87.68 (69.43)                    |
| Neem cake           | *Azadirachta indica* A                | Meliaceae           | Cake            | 10.41                                               | 88.42 (70.08)                    | 9.08                                                 | 89.90 (71.45)                    |
| Piper nigrum        | *Piper nigrum*                        | Piperaceae          | Dried Unripe Fruit | 15.33                                           | 82.96 (65.59)                    | 14.58                                               | 83.79 (66.24)                    |
| Control             |                                       |                     |                 | 90.00                                               | 0.00 (4.05)                      | 90.00                                               | 0.00 (4.05)                      |

*Mean of three replications, Figures in the parentheses are angular transformed values

3014
**Fig.1** Pure culture of *Fusarium oxysporum* f.sp. *ricini*

**Fig.2** Botanicals used in the *in vitro* study against castor wilt pathogen
Fig. 3 Plant extracts prepared for the study
**Fig. 4** *In vitro* evaluation of plant extracts at 5% concentration against *F. oxysporum f.sp. ricini*
Fig. 5 In vitro evaluation of plant extracts at 10% concentration against *F. oxysporum* f.sp. *ricini*
Fig. 6 Effect of plant extracts on the radial growth of *F. oxysporum f.sp. ricini*
In current study, a bunch of botanicals which possess fungitoxic properties against *F. oxysporum* f.sp. *ricini* has been discovered, include neem cake, henna, aloe, ashoka, tulsi, eucalputus bark, neem bark, others presented in Table 1. Moreover, plant extracts have equal potential as fungicides for the reduction of pathogen growth, as observed in the results. The results are in accordance with Irum, 2007, who reported antifungal effect of aqueous extracts of four plant species viz., *Azadirachta indica*, *A. Juss.*, *Datura. metel* L., *Ocimum sanctum* L. and *Parthenium hysterophorus* L., found that all the four plant extracts tested at 40 percent concentration effectively reduced the mycelial growth of *F. oxysporum* f.sp. *ciceri*.

Moreover, interesting fast noted in the study was, concentration of plant extracts used is directly proportional to the inhibition of pathogen growth, implies higher the concentration of botanical, higher the pathogen growth inhibition. Additionally, in twenty-six botanicals used, the plant parts found to be having promising fungitoxic against wilt pathogen include neem cake powder, leaves (henna, ashoka, tulsi, mint, calotropis), bark (neem and eucalyptus) and dried fruit (black pepper) etc.

In conclusion, our investigation put forth, that neem cake, henna leaves and aloe are the best botanicals among others tested plant species in inhibition of *F. oxysporum* f.sp. *ricini* growth. In the plant species tested, the plant parts that could be effective in inhibition of castor wilt pathogen mostly include leaves, bark etc. Also, the above tested best three botanicals neem cake, henna and aloe could be easily procured by the farmers from the local market and could be used for the management of the castor wilt disease at lower cost. Furthermore, season long market availability of these three botanicals, provide easy access and would be economical for the farmers. Additionally, botanical based protection is ecofriendly and sustainable. However, still further research need to be done to better understand the tested botanicals mechanisms against the wilt pathogen; Future line of research may include efficacy of these botanicals at glass-house and field conditions; Isolation and identification of individual compounds from the plant crude extracts and mode of action against the castor wilt pathogen.

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