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COMPATIBILITY OF DIFFERENT OILS WITH *BEAUVERIA BASSIANA*, A POTENTIAL ENTOMOPATHOGENIC FUNGUS

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ABSTRACT
Beauveria bassiana, commonly known as white muscardine fungus is considered to be an potential entomopathogen.Formulation and application techniques play a significant role in success of entomopathogens in field circumstances. Oil formulation of entomopathogens may be promoted for their enhanced infectivity, prolonged persistence and increased shelf life. The compatibility of six oils *viz*., Neem, Pungam, Castor, Mahua, Corn and Paraffin were investigated under *in vitro* condition by following poison food technique, to explore suitable oil based carrier for *Beauveria bassiana* (Bb 112). The vegetative growth and sporulation were recorded on10th and 15th day after inoculation and compatibility of oils were worked out. Among the oils tested, Neem, Castor, Mahua, Corn, Paraffin at1, 2, 3, 5 and 10 percentage were compatible with *Beauveria bassiana*. Pungam oil was found to be not compatible (T factor - 7.90). Paraffin oil at 10 percentage recorded highest colony diameter (6.50 cm) to control (8.70 cm).Regarding the sporulation, fungus sporulated well in corn oil at 10 percentage (1.83 x 10^{8} spores / mL) followed by paraffin oil (1.80 x 10^{8} spores /mL). Even though all the oils were compatible except pungam oil, paraffin oil may be encouraged, because of their rheology that may suit well for formulation

INTRODUCTION

Fungal pathogens particularly, *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metschinkoff), *Verticillium lecanii* (Zimmerm.) Zare and Games *Nomuraea rileyi* (Farlow) Samson had been found to be promising in the control of several agricultural pests (Lingappa *et al.,* 2005). *B. bassiana* is a hyphomycete insect pathogenic fungus in the subdivision Deuteromycotina which occurs worldwide. Over 200 species of insects in nine orders, mainly Lepidoptera and coleoptera had since been recorded as hosts (Li and Yang, 1988). It is found naturally on some plants and in soils and is regarded as a safe biopesticide (Uma Devi *et al.,* 2008). The appropriate use of environment-friendlymicrobial pesticides can play a significant role in sustainable crop production by providing a stable pestmanagement program. Because, biological control is generally perceived as providing both long-lasting insect control and having less potential for damage to the environment or non-target organisms than chemical interventions (Grace, 1997; Hokkanen and Lynch, 1995;Howarth, 1991; Khetan, 2001; Bhadauria *et al.,* 2012). Many of the currently available formulation of entomopathogens are wettable powders containing conidia. Water is a useful formulating agent because it is non - toxic, readily available, cheap and can be dispersed using simple hydraulic sprayers. However, the future thrust for development of mycopesticides has been promoted by the discovery that fungal conidia formulated in oils have shown greater efficacy than conventional water-based suspensions. The asexual spores of entomopathogenic fungi (*Beauveria, Metarrhizium,Nomuraea* and *Paecilomyces*) posess hydrophobic cell walls containing a rodlet layer composed of cysteine rich glycoprotein termed as hydrophobins, which make them amenable to formulate in oil. Oil formulations has been reported to increase the adhesion of propagules to the insect integument, enhance spread of inoculums over the insect body, enhance penetration of insect cuticle, protect propagules from ultraviolet radiation and enhance infection under low humidity (Inglis *et al.,* 2002).Prerequisites of ecofriendly and sustainable pest management agenda consists of combination of management tactics like use of aqueous extracts and oils from plants, agriculture and horticulture mineral oils, biocontrol agents, newer chemical molecules etc., to achieve cost effective integrated pest management program There are many reports which describes the compatibility of *B. bassiana* with neem products (Gupta *et al.*, 1999; Hirose *et al.,* 2001; Depieri *et al.,* 2005). Furthermore compatibility studies with petroleum based and vegetable oils would help to reveal the possibilities of selecting appropriate oil carrier for formulation.Hence, in present study, the fivedifferent types of oils along with corn oil as a standard were selected in view to select the oils for formulating *B. bassiana* (Bb 112) conidia that may help to achieve synergestic result in pest management program.

MATERIALS AND METHODS

Six types of oils viz., Neem, Pungam, Castor, Mahua ,Corn, Paraffin that are commonly available at cheaper cost were selected for experimental purpose. The strain isolated from larvae in paddy (Bb 112) (Location: Coimbatore, Tamil Nadu, India) was used for investigation. Poison food technique

(Olmert and Kenneth, 1974) was followed where SMAY (Sabouraud's Maltose Yeast Agar) media was amended with six oils @ 1, 2, 3, 5 and 10 percent concentration each. Unamended SMAY media served as control. The treatments were replicated four times each. The media were poured in to 90 mm petridishes for solidification. The well sporulated culture grown on SMAY for 15 days was cut into 6 mm disc by using cork borer. The cut block was transferred to SMAY + oil amended media and kept in inverted position by using inoculation needle. The plates were kept for incubation at 27±1° C, 65-70 % RH in BOD incubator.

Determination of treatments effect on mycelia growth and sporulation

The vegetative growth of colony was measured in cm on 5th and 10th day. The measurement was taken in two directions at right angle to each other and the average of two was taken.
The conidial concentration was estimated on 15th day after inoculation from the surface of the mycelium covering the petriplates. To prepare the spore suspension, the conidia were harvested by flooding the plates with 0.05 % Tween 80 (Astron chemicals Pvt Ltd.,) along with 10 mL sterile water. Tween 80 serves the purpose of wetting agent. The resulting suspension were stirred in magnetic stirrer for 5 minutes and filtered through sterile muslin cloth to eliminate the coagulated medium from petriplate. By using an improved Neubauer ruled haemocytometer and phase contrast microscope at a magnification of 400x, the spore yield was recorded and the results were expressed in number of spores per mL to determine the effect of oils.

Compatibility calculation

Compatibility was calculated by using the formula proposed by Alves *et al.* (1998) to classify chemical products according to their toxicity to entomopathogenic fungi *in vitro*.

This classification is based on calculations of the T factor in whichthe observations on vegetative growth and sporulation were transferred relative to the control (100) and the product's toxicity (T) values was calculated as per the following formula

T = [20 (VG) + 80 (SP)]/100.

T values between 0 and 30 classify products as very toxic; from 31 to 45 as toxic; from 46 to 60, moderately toxic and above 60, products are considered compatible with the fungus being studied.

Statistical analysis

The experiment was conducted in a controlled randomized design (CRD). The data obtained on mycelia growth was pooled and made an average from all four replicates and subjected to one way ANOVA. The treatment means were compared using critical difference (CD) at p = 0.05.

RESULTS

In the present study six types of oils were investigated for its compatibility with *B. bassiana* under *invitro* condition. The significant difference in colony diameter was observed between the treatments. The control plates recorded the growth with highest colony diameter (4.50cm) on 5th day. Among the treatments, at 1 % concentration, on 5th day mahua oil amended plates showed highest mycelia growth (4.00 cm) next to control which was followed by corn oil treated plates (3.10 cm). Addition of paraffin oil with fungus resulted in 3.00 cm diameter colony and was on par with castor oil amended media (3.00 cm). This was followed by neem oil amended media (2.60 cm). Pungam oil treated media recorded least mycelia growth with colony diameter of 2.00 cm. The significant variation in mycelia growth could be observed with respect to time and concentration. On 10th day, castor oil treated plates recorded the highest colony diameter (8.70 cm) and was on par with control. Followed by castor oil, the colony diameter was high in neem oil and paraffin oil treated media (8.50 cm). The corn oil treatment registered colony diameter of 8.60 cm and was found to be on par with control, neem oil and paraffin oil treatments. This was followed by mahua oil with colony diameter of 7.75 cm and pungam oil recorded the least growth 0f 5.00 cm mycelia growth.

At 2 % concentration on 5th day, mahua oil treatment ascertained 3.80 cm colony diameter. This was followed by corn oil amended media with 3.40 cm colony growth. Paraffin oil treatment recorded colony diameter of 2.80 cm followed by castor oil amended media (2.60 cm). Addition of neem oil with fungus recorded 2.40 cm colony diameter followed by pungam oil amended media (2.00 cm). On 10th day the mycelia growth of 7.80, 7.75, 7.60, 7.55 cm was observed in Neem, paraffin, corn and castor oil respectively and found to be on par with each other. There was no significant growth difference within 5 days unlike other oils,in pungam oil treated fungus (2.95 cm).

At 3 % concentration, almost similar trend was observed with minor variations. On 5thday, mahua oil showed highest colony growth among the treatments (2.70 cm). The colony diameter of 2.50,2.46, 2.32, 2.30, 1.80 cm was recorded in castor,

Table 1: Effect of different oils on the mycelial growth of *B. bassiana*

Treatments	Colony diameter in 'cm' (Cumulative)*									
	1%		2%		3%		5%		10%	
	5th day	10th day	5th day	10th day	5th day	10th day	5th day	10th day	5th day	10th day
Neem oil	2.60^{e}	8.50^{b}	2.40^{f}	7.80^{b}	2.30^{d}	7.30^{d}	2.20^{f}	6.20^{d}	2.20^{c}	6.00^{d}
Pungam oil	2.00^{f}	5.00^{d}	2.00^{g}	2.95^{e}	1.80^{e}	2.18^{f}	1.00^{g}	2.00^{f}	1.00^{e}	1.75^{f}
Castor oil	3.00^{d}	8.70^{a}	2.60^{e}	7.55^{c}	2.50^{c}	7.45^{d}	2.50^{d}	6.80^{b}	1.20^{d}	6.25^{c}
Mahua oil	4.00^{b}	7.75^{c}	3.80^{b}	6.30^{d}	2.70^{b}	5.45^{e}	2.61^{c}	5.40^{e}	2.40^{b}	5.00^{e}
Corn oil	3.10^{c}	8.60^{ab}	3.40^{c}	7.60^{bc}	2.32^{d}	8.00^{c}	2.35^{e}	6.40^{c}	2.35^{b}	6.15^{cd}
Paraffin oil	3.00^{d}	8.50^{b}	2.80^{d}	7.75^{bc}	2.46^{c}	8.25^{b}	3.00^{b}	6.45^{c}	2.40^{b}	6.50^{b}
Control	4.50^{a}	8.70^{a}	4.50^{a}	8.70^{a}	4.50^{a}	8.70^{a}	4.50^{a}	8.70^{a}	4.50^{a}	8.70^{a}
CD = 0.05	0.09	0.18	0.09	0.21	0.07	0.19	0.07	0.14	0.08	0.16

*Mean of 4 replications; Means in the same column with letters in common are not significantly different

Table 2: Compatibility (T factor) of *B.bassiana* with different oils

Treatments	1% VG	SP	T*	2% VG	SP	T*	3% VG	SP	T*	5% VG	SP	T*	10% VG	SP	T*
Neem oil	85.00(97.70)	1.73(83.98)	86.72(D)	78.00(89.66)	1.81(87.86)	88.22(D)	73.00(83.91)	1.81(87.86)	87.07(D)	62.00(71.26)	1.8(87.38)	84.15(D)	60.00(68.97)	1.79(86.89)	83.30(D)
Pungam oil	50.00(57.47)	0.27(13.11)	21.98(A)	29.00(33.33)	0.18(8.74)	13.65(A)	21.80(25.06)	0.11(5.34)	9.28(A)	20.00(22.29)	0.1(4.85)	8.33(A)	17.50(20.11)	0.1(4.85)	7.90(A)
Castor oil	87.00(100.0)	1.94(94.17)	95.33(D)	75.00(86.21)	1.76(85.44)	85.59(D)	74.50(85.63)	1.8(87.38)	87.03(D)	68.00(78.16)	1.84(89.32)	87.08(D)	62.50(71.84)	1.83(88.83)	85.43(D)
Iluppai oil	77.00(88.51)	1.88(91.26)	90.71(D)	63.00(72.41)	1.64(79.61)	78.17(D)	54.50(62.64)	1.78(86.41)	81.65(D)	54.00(62.07)	1.85(89.81)	84.26(D)	50.00(57.47)	1.81(87.86)	81.78(D)
Corn oil	86.00(98.85)	1.75(84.95)	87.73(A)	76.00(87.35)	1.82(88.35)	88.15(D)	80.0(91.95)	1.83(88.83)	89.45(D)	64.0(73.56)	1.75(84.95)	82.67(D)	61.5(70.69)	1.83(88.83)	85.20(D)
Paraffin oil	85.00(97.70)	1.83(88.83)	90.60(D)	77.5(89.08)	1.85(89.81)	89.66(D)	82.5(94.82)	1.72(83.50)	85.76(D)	64.5(74.13)	1.71(83.01)	81.23(D)	65.0(74.71)	1.80(87.38)	84.84(D)
Control	87.00(100.0)	2.06(100.0)	-	87.00(100.0)	2.06(100.0)	-	87.00(100.0)	2.06(100.0)	-	87.00(100.0)	2.06(100.0)	-	87.00(100.0)	2.06(100.0)	-

VG – Vegetative growth (mm), SP – Sporulation (x 10^8 spores ml^{-1}); Values in parenthesis are values relative to control (100); *Classification based on the formula T = (20 VG + 80 SP) / 100; A – Very toxic ; D- Very Compatible

paraffin, corn, neem and pungam oil treatments. Corn and neem oil amended media were on par with each other. On 10th day, 8.25, 8.00. 7.45, 7.30, 5.45, 2.18 cm colony diameter was noted in paraffin, corn, castor, neem, mahua and pungam oil amended media. Neem oil and castor oil amended treatments were found to be on par with each other.

At 5 % concentration, unlike in 1, 2 and 3 % concentrations, on 5th day , paraffin oil amended media recorded highest colony diameter (3.00 cm) , followed by mahua oil treatment (2.61cm), castor oil amended media (2.50 cm), corn oil treatment (2.35 cm), neem oil (2.20 cm) and pungam oil (1.00 cm). On 10th day,castor oil amended media showed highest mycelia growth (6.80 cm) next to untreated control. The mycelia growth of 6.45 and 6.40 cm was noted in paraffin and corn oil amended media and both treatments were found to be on par with each other. This was followed by neem oil amended media with colony growth of 6.20 cm. Mahua oil treated media recorded colony growth of 5.40 cm diameter and pungam oil recorded least growth (2.00 cm)

At 10 % concentration, three treatments, viz., paraffin, mahua and corn oil treated plates showed highest mycelia growth next to control (2.40, 2.40 and 2.35 cm respectively) and three treatments were found to be on par with each other. This was followed by neem oil treatment (2.20 cm), castor oil (1.20 cm) and pungam oil treated fungus (1.00 cm). On 10th day, paraffin oil treated fungus showed highest mycelia growth of 6.15 cm. The colony diameter of 6.25, 6.15 and 6.00 cm were observed in castor, corn and neem oil treated fungus and these three treatments were found to be on par with each other. Mahua oil treated fungus follows the next by recording mycelia growth of 5.00 cm and pungam oil recorded least growth (1.75 cm). (Table 1)

Regarding sporulation, all oils except pungam oil , irrespective of concentration, recorded well spore count ranging from 1.71 – 1.94 x 10^8 spores / mL.. However the control plate was found to be high by showing 2.06 x 10^8 spores / mL. Pungam oil amended plates accounted for least sporulation within the range of 0.1 – 0.27 x 10^8 spores/ mL at five concentrations.

Based on colony diameter in cumulative and sporulation, combatibility index (T * value) was calculated. All the treatments irrespective of concentrations, was found to be highly compatible with Compatibility index as (D) (T * value range: 78.17 - 95.33). Pungam oil was not compatible with Combatibility index as (A)(T* value range: 7.90 - 21.98) (Table 2).

DISCUSSION

In the present investigation it can be clearly depicted that all the oils (Neem, castor, mahua, corn and paraffin oils) are found to be highly compatible with *Beauveria bassiana* strain used in this study, except pungam oil. The deep insight into biological advantages and disadvantages of oil carrier, helps to explore new paradigms that may enhance efficacy of oil based formulations. Mechanisms proposed for synergistic activity of oils with entomopathogenic fungi include enhanced adhesiveness of conidia to the lipophilic insect cuticle, disruption (solubilization) of cuticular waxes leading to improved deposition (e.g., by carrying conidia to thin

intersegmental membranes) and improved secondary acquisition of fungal inoculum from vegetation or other sprayed surfaces. (Boucias *et al.*, 1991; Ibrahim *et al.*, 1991; Inglis *et al.*, 2002) It is also hypothesized that at non-lethal doses, oils might induce physiological stresses to insects that predispose them to infection. (Avery *et al.*, 2013).

The present results are in agreement with Mohan *et al.* (2007) who reported that 30 isolates of *B. bassiana* with a commercial neem formulation (0.3% v/v) and although conidial germination was delayed in all isolates, it was generally not significantly decreased and 23 of the isolates were considered to be compatible. However, some studies report that *in vitro* growth of *B. bassiana* (germination, colony formation and sporulation) is negatively affected by emulsifiable neem oils. Hirose *et al.* (2001) revealed that Neem oil (AR) @ 2% promoted a larger negative effect on *B. bassiana*, inhibiting germination (-45.27%), colony diameter (-36.62%) and conidiogenesis (-84.93%).

The non compatibility of pungam and castor oil with *Paecilomyces farinosus* was reported by Vishalakshy *et al.* (2006) who depicted that these oils though enhanced mycelia growth, caused a significant reduction in sporulation. This is in contradictory with present investigation that pungam oil is totally incompatible and castor oil amended media enhanced mycelia growth and sporulation. This may be due to variability in susceptibility to oils between entomopathogenic fungus.

Corn oil is generally composed of several unsaturated fatty acid including linoleic acid, oleic acid and very small amount of saturated fatty acids. Such unsaturated fatty acids have very high boiling points and stabilize cell metabolisms of fungal conidia against unfavourable environmental abiotic factors (Nyam *et al.*, 2009.). The suitability of corn oil as an carrier was reported by Luz and Batagin (2005) as more than 25 percent conidial germination in 10 % corn, thistle and linseed oil, after 8 days of incubation.

Eventhough results from the present experiment shows that all the oils are compatible except pungam oil, the notable observation is that at all concentration, on 10th day of observation paraffin oil treated media records highest mycelia growth next to control. This finding also coincides with the studies of Avery *et al.* (2013), whose studies provide information that

I. fumosorosea growth *invitro* were reduced least by petroleum-based materials and most by botanical oils, borax, and some ofthe copper-based fungicides. This may be due to the fact that plant oils are more subject to chemical alterations, such as oxidation, which can be very detrimental to fungal propagules. Furthermore oxidation (= rancidity) of plant oils (particularly polyunsaturated fatty acids causes the formation of volatile compounds such as ketones, aldehydes and low – molecular weight fatty acids which can be very toxic (Kabara *et al.*, 1977). Although the inclusion of antioxidants (eg. Butylated hydroxyanisole and Butylated hydroxytoluene) can negate the adverse impact of free radicals, many antioxidants are antimicrobial themselves. (e.g. Phenolic based). (Inglis *et al.*, 2002). However the volume of oil required providing effective coverage in many cases would also be phytotoxic.

The primary advantage of petroleum based material (paraffin oil) is consistency of viscosity over broad range of temperatures, availability and stability. These characteristics can be well correlated with studies of Akhtar *et al.* (2009) who depicted the rheology of different vegetable oils including paraffin oil. The results showed that, paraffin oil containing no fatty acids has low viscosity (7.11 m Pas.s) and possess the maximum spreadability with 17.5 mm spreading diameter than vegetable oils. Hence petroleum based material rather than vegetable oils may be well preferred as carrier material for oil based formulation of mycopesticides.

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