Safety Evaluation of Field Recommended Insecticides on European Honeybee (*Apis mellifera*) Under Laboratory Conditions

ABSTRACT

A laboratory experiment was conducted to assess the safety of different insecticides *Viz.,* chlorpyriphos 20 EC, profenofos 50 EC, lambda-cyhalothrin 5 EC, thiamethoxam 25 WG, acetamiprid 20 SP, *Beauveria bassiana*, neem oil, and pongamia soap) at their field-recommended doses to the European honey bee, *Apis mellifera*, using the dry film method. Foraging bees were released into the treated jars at different intervals *i.e.,* 0, 12 and 24 hhours after dry film formation. Chlorpyriphos 20 EC, profenofos 50 EC, thiamethoxam 25 WG, acetamiprid 20 SP, lambda-cyhalothrin 5 EC, and pongamia oil caused high mortality rates across different exposure intervals. In contrast, neem oil 1500 PPM exhibited low to moderate toxicity, while *Beauveria bassiana* was found to thbe least toxic to *A. mellifera* indicating its suitability in pollinator-friendly pest management programs.

Keywords: Honey bee, *Apis mellifera*, toxicity, mortality, dry film.

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1. INTRODUCTION

Modern agriculture has become increasingly reliant on agrochemicals, including fertilizers, pesticides, herbicides, and fungicides, to meet ~~the demands of~~ global food production (Sattari *et al.,* 2016; Wu *et al.,* 2023 and Chreil and Maggi, 2023). Approximately 5.6 billion pounds of ~~various~~ agrochemicals are used in modern agriculture (Zhao *et al.,* 2022). However, the use of agrochemicals is projected to increase in the coming years because~~due~~ of~~to~~ rising global food demands and intensification of agricultural practices, which would further increase the potential risks to non-target organisms, especially pollinators, such as honey bees (Brittian *et al.,* 2010; Ratnakar *et al*., 2016 and Migdal *et al.,* 2018).

Bees provide pollination services to various food crops and~~as well as~~ wild plants (Klein *et al.,* 2007; Pashte and Said, 2015). Bees are reliable pollinators~~,~~ because~~as~~ they visit flowers systematically to collect nectar and pollen. It is estimated that 80 per cent of insect pollination ~~by insects~~ is performed~~done~~ by bees (Hoehn *et al.,* 2008). Honeybees (*Apis mellifera*) play a vital role in pollination and~~,~~ contribut~~cing~~ significantly to the productivity and sustainability of many agricultural and horticultural crops. However, the indiscriminate use of chemical insecticides in~~for~~ pest management poses a potential threat to these beneficial pollinators. Recently, declines in~~of~~ various pollinators have been reported worldwide (Potts *et al.,* 2010 and Cameron *et al.,* 2011). While many environmental and human-related factors are still being studied in relation to yearly honey bee colony losses, pesticides have~~are~~ consistently been identified as a major cause (Smith *et al.,* 2014).

Cashew (*Anacardium occidentale* L.) is an important plantation crop cultivated in the tropical and subtropical regions of India, including Andhra Pradesh, Kerala, Karnataka, Goa, Maharashtra, Tamil Nadu, and Odisha. The crop is highly susceptible to several insect pests, notably the tea mosquito bug (*Helopeltis antonii*), leaf miner (*Acrocercops syngramma*), ~~and~~ stem and root borer (*Plocaederus ferrugineus* L.),Thrips, Mealy bug ~~etc~~. These pests cause severe economic losses by damaging tender shoots, inflorescences, and nuts, ultimately affecting the yield and nut quality. To manage these pests, farmers commonly use insecticides belonging to diverse chemical groups, such as neonicotinoids, organophosphates, pyrethroids, and newer molecules, such as~~like~~ diamides and spinosyns. However, the frequent and often indiscriminate

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use of such chemicals can pose varying ~~degrees of~~ risks to non-target pollinators visiting cashew flowers.

Studying the effects of pesticides on honey bees is crucial~~,~~ as it enables the control of insect pests while minimizing harm to bees that come into contact with these chemicals during foraging (Pashte and Patil, 2017). Loss of honey bees ~~will~~ directly affects honey production and indirectly affects crop production due to insufficient pollination. The non-target impact of insecticides on honey bees can lead to excessive sub-lethal effects, direct mortality, and repellent behavio~~u~~r, as well as toxic residues on the floral parts and nectar of crops (Halm *et al.,* 2006 and Desneux *et al.,* 2007). Honey bee behavio~~u~~r, such as communication dances, return flights, orientation, and foraging efficacy during floral visits, are ~~getting~~ affected when it is ingets direct contact with insecticides or insecticide-treated floral parts during insecticide application (Vandame *et al.,* 1995; Abrol and Kumar, 2009 and Deepika *et al.,* 2022).

Assessing the safety of insecticides on pollinators is essential for developing sustainable pest management strategies that safeguard the ecological balance. Laboratory bioassays under controlled conditions provide an effective means to evaluate the acute toxicity and behavioral effects of insecticides on honeybees at field-recommended concentrations. Such studies are critical for identifying bee-safe molecules and for guiding integrated pest and pollinator management programs in cashew ecosystems.

The present study was undertaken to evaluate the safety of field-recommended insecticides used in cashew cultivation against the European honeybee (*Apis mellifera*) under laboratory conditions, with the objective of identifying insecticides that are comparatively safe for~~to~~ pollinators.

2. MATERIALS AND METHODS

~~In order~~ T~~t~~o identify insecticides that are safer to honeybees, ~~selected~~ newer insecticide molecules and bio-pesticides commonly used in cashew pest management were evaluated for their toxicity against the European honeybee (*Apis mellifera*) under laboratory conditions. The experiment was conducted from~~during~~ 2023 to ~~–~~2024 at~~in~~ the Department of Entomology, Agricultural College, Bapatla. Forager honeybees were collected at the entrance of the hives using a~~cone~~ cone-type muslin hand net (30 cm diameter) and transferred into plastic jars. The collected bees were immobilized by

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keeping them in a refrigerator for approximately five minutes following the method described by Sharma and Abrol (2005), and subsequently allowed to recover from cold treatment before exposure to insecticide treatments. They were identified by their slightly larger body size, wear-outworn out wings, hairier bodies, and well--developed pollen baskets on their hind legs.

Toxicity assessment was performed by exposing a definite number of bees (n = 10/ treatment/replication) to a thin dry film of insecticide inside the glass jars. The experiment was laid out in a Completely Randomized Design (CRD) with three replications and nine treatments, namely: chlorpyrifos 20 EC, profenofos 50 EC, lambda--cyhalothrin 5 EC, thiamethoxam 25 WG, acetamiprid 20 SP, *Beauveria bassiana*, neem oil, pongamia soap, and an untreated control.

The toxicity of tThe test insecticides to honeybees waswere evaluated usingfor thetheir toxicity to honey bees by dry film method. In this method, the recommended doses of the insecticide solutions were prepared by diluting the required quantity of the commercial formulation in one litre of distilled water. One millilitermlml of each of the insecticide solutions was applied as a thin film inside the walls of a clean dry rearing jar of size 10 × 7 cm in diameter, and the jar was gently rotated and left for drying so that a thin dry film of the chemical was formed inside the jar.

Ten numbers of foraging bees were released into the each treated jar at different intervals *i.e.*, 0, 12 and 24 hhours after dry film formation. Since bees were exposed to insecticides at any time after their spraying in the field, these intervals were considered to understand the residual toxicity of the insecticides. A cotton pad soaked in sugar solution (20%) was provided inside the jar as food for bees, and the jar was covered withusing a muslin cloth. Each treatment for bees was replicated three times,thrice and a jar with a dry film of distilled water alone was considered as the untreated control.

Mortality counts: The mortality of the honey bees waswere recorded at 0,2, 4, 6, 12, 24, and 48 hhours after release into the jars, and theper percent mortality was calculated. The bees were considered as dead only when gentle pressure upon the abdomen did not bring any twitching movement or a positive response over a half-- an hour inspection period. Abbotts's correction (1925) was applied if mortality occurreds in the control grouptreatment. The mortality values were transformed to arc-sine values and then analyzed using the SPSS software. Grouping of means was done by DMRT at P=0.05 (Gomez and Gomez, 1984).

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Abbott's formula

$$\text{Per cent corrected Mortality} = \frac{\text{Per cent mortality in treatment} - \text{Per cent mortality in control}}{100 - \text{Per cent mortality in control}} \times 100$$

3. RESULTS AND DISCUSSION

Safety of Recommended Dose of Insecticides to *A. mellifera* Honey Bees Exposed Immediately after Dry Film Formation: Among all the treatments, chlorpyriphos 20 EC and thiamethoxam 25 WG showed percent per~~-~~cent mortality after two hours followed by profenofos 50 EC, lambda~~-~~-cyhalothrin 5 EC, and pongamia soap, which showed cent per~~-~~cent mortality after four hours of release of honey bees. Acetamiprid 20 SP showed mortality rates of 33.33%, 50%, and 66.67% at two, four and six hours after release~~, of honey bees~~ respectively, but showed a percentage of~~per cent~~ mortality after 12 h~~hours of release~~ of honey bee release~~bees~~. Bees treated with neem oil 1500 PPM showed constant increases in mortality *i.e.,* 26.67, 50, 60%, and 64.07 per cent after two, four, six and 12 h~~hours~~ of release, respectively, and decreased thereafter at 24 and 48 h~~hours~~ with 61.11% and 56.55% mortality, respectively. *Beauveria bassiana* exhibited a mortality of 3.33% after 12 h~~hours~~ of release and increased thereafter at 24 and 48 h,~~hours~~ with 11.11% and 61.31% mortality, respectively (Table 1).

Safety of Recommended Dose of Insecticides to *A. mellifera* Honey Bees Exposed 12 hours after Dry Film Formation: Thiamethoxam 25 WG showed ~~cent~~ per~~-~~cent mortality after four hours followed by chlorpyriphos 20 EC, which showed ~~cent~~ per~~-~~cent mortality after 12 h~~hours~~ of release of honey bees. Both lambda~~-~~-cyhalothrin 5 EC and acetamiprid 20 SP showed a constant increase in mortality from two hours to 12 h~~hours~~ of release and then showed ~~cent~~ per~~-~~cent mortality after 24 h~~hours~~. Profenofos 50 EC also showed a constant increase in mortality from 16.67% at two hours of release to 92.13% at 24 h~~hours~~ of release and showed ~~cent~~ per~~-~~cent mortality at 48 h~~hours~~ of release. Neem oil 1500 PPM recorded high mortality (51.85%) at 24 h~~hours~~ and then decreased at 48 h,~~hours~~ with 47.62% mortality. There was no record of mortality in

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bees treated with *Beauveria bassiana* and P.~~pongamia~~ soap for upto four hours but a sudden rise in mortality of 17.41 and 3.33 per cent, 39.26 and 3.33 per cent, 36.11 and 7.41%, ~~per cent~~ and 43.45 and 42.26 per cent at six, 12, 24, and 48 h~~hours~~, respectively (Table 2).

Safety of Recommended Dose of Insecticides to *A. mellifera* Honey Bees Exposed 24 hours after Dry Film Formation: Among all the treatments, chlorpyriphos 20 EC showed percent per-cent mortality after six hours followed by thiamethoxam 25 WG and acetamiprid 20 SP, which showed cent per-cent mortality after 12 h~~hours of release~~ of honey bee release~~bees~~. Profenofos 50 EC, neem oil 1500 PPM, and P~~p~~ongamia soap showed constant increases in mortality of 16.67%, 13.33%, and 3.33% at two hours of release, with~~to~~ the highest mortality rates of 91.07%, 45.24%, and 91.07% at 48 h~~hours~~ of release, respectively. Lambda cyhalothrin 5 EC did not record any mortality after two hours but showed 6.67%, 37.03%, and 76.39% at four, six and 12 h~~hours~~ after release of honey bees, respectively, and the~~n cent~~ per-cent mortality after 24 h~~hours~~. *Beauveria bassiana* recorded a mortality of 39.29% after 24 h~~hours~~ and then decreased slightly, with a mortality of 36.31% after 48 h~~hours~~ (Table 3).

Among organophosphates~~organophospahtes~~, chlorpyrif~~ph~~os 20 EC was found to be highly toxic to *A. mellifera* resulting in mortality rates ranging from 63.33% to 100.00% after 2 h~~hours~~ of exposure. Another organophosphate insecticide, P~~p~~rofenofos 50 EC, caused mortality ranging from 33.33 to 100.00 per cent in *A. mellifera* after 4 h~~hours~~ of exposure. Similar results were reported by Reddy and Reddy (2006) and Stanley *et al.* (2015), who found that the majority of organophosphates were highly toxic to honeybees. These results also corroborate ~~with~~ the findings of Leite *et al.* (2021) and Deepika *et al.* (2022), who reported maximum mortality of honey-bees in chlorpyriphos 20EC and profenophos 50EC treatments, respectively.

Among neonicotinoids, thiamethoxam 25 WG was found to be highly toxic to *A. mellifera*, with ~~recording~~ a mortality of 76.67% to 100% after 2 h~~hours~~ of exposure. In the case of another neonicotinoid group member, acetamiprid 20 SP was also found to be moderately to highly toxic, with ~~recording~~ a mortality of 85.93% to 100% in *A. mellifera* after 12 h~~hours~~ of exposure. This result is consistent~~was in concurrence~~ with those~~the results~~ of Bailey *et al.* (2005) and Hasansab *et al.* (2013), who reported that neonicotinoids were moderately to highly toxic to honey bees. Danielle et al. (2009), Costa et al. (2013), and Stanley et al. (2015) also reported that~~,~~ regardless of how the

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bees were exposed to insecticides, thiamethoxam was highly toxic to *A. mellifera*. The findings of Ratnakar *et al.* (2016), who reporstated that thiamethoxam caused the highest mortality (80%) in *A. mellifera* when the bees were exposed 12 hhours after dry film formation, also support the present findings of the study.

Among synthetic pyrethroids, lambda- -cyhalothrin was found to be highly toxic to *A. mellifera* causing 60.74% to 100.00% mortality after 12 hhours of exposure. These results arederive supported by those offrom Bailey *et al.* (2005), who found that lambda-cyhalothrin was highly toxic to *A. mellifera*. A recent study conducted by Deepkia *et al.* (2022), who stated that lLambda- -cyhalothrin 5EC @ 0.6 ml/ l caused a mortality of 45.92 and 68.06% into *A. cerana indica* and *T. iridipennis*, respectively, also supports the present findings of the study.

Among the botanicals, neem oil 1500 PPM was found to be low to moderately toxic to *A. mellifera* causing 23.33% to 50.00% mortality after 4 hhours of exposure. In contrastOn the other hand, Ppongamia soap was found to be highly toxic to *A. mellifera* with 42.26% to 100.00% mortality after 48 hhours of exposure. Thes- above results awere in line with the findings of Cunha Pereira *et al.* (2020) and Vagare *et al.* (2024) that spraying of neem oil was repellent to honey bees duringat the initial hours after spraying and concluded that the botanicals werewas safe for honey bees.

Among microbial pesticides, *Beauveria bassiana* was lessfound low toxic to *A. mellifera* with 11.11% to 39.29% mortality after 24 hhours of exposure. Studies conducted by Ahmed and Abd-Elhady (2013), Potrich *et al*. (2018), and Gencer and Bayramoglu (2022) also reported low toxicity of *Beauveria bassiana* to honey bees.

4. CONCLUSION

InFrom the present investigation, it was revealed that the organophosphates (chlorpyriphos 20 EC and profenofos 50 EC), neonicotinoids (thiamethoxam 25 WG and acetamiprid 20 SP), the pyrethroid insecticide (lambda-cyhalothrin 5 EC), and pongamia soap were found to be highly toxic to *Apis mellifera*, causing 100% mortality within the observation period under laboratory conditions using the dry film method. In contrast, neem oil (1500 ppm) and the entomopathogenic fungus, *Beauveria bassiana* were found to be lesslow in toxicity and comparatively safer forto *A. mellifera*, when applied at recommended doses. This indicates their potential suitability as pollinator-safe alternatives in integrated pest management (IPM) programs, and which havethe dry

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~~film method~~ proven~~d~~ effective in detecting acute toxicity under controlled conditions and can serve as a useful tool for pre-screening pesticide safety i~~o~~n pollinators.

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Table 1. Effect of insecticides at their field recommended doses on mortality of European honey bee, *Apis mellifera L.* exposed Immediately after treatment (Zero hours)

Treatments		Dosage (ml or g/L)	Mean Corrected mortality (%)						
			0 h	2 h	4 h	6 h	12 h	24 h	48 h
T_1	Chlorpyriphos 20 EC	2.5 ml/L	0.00 (0.91)	100 (89.09)a	100 (89.09)a	100 (89.09)a	100 (89.09)a	100 (89.09)a	100 (89.09)a
T_2	Profenofos 50 EC	1 ml/L	0.00 (0.91)	76.67 (61.22)b	100 (89.09)a	100 (89.09)a	100 (89.09)a	100 (89.09)a	100 (89.09)a
T_3	Lambda cyhalothrin 5 EC	1 ml/L	0.00 (0.91)	56.67 (48.93)c	100 (89.09)a	100 (89.09)a	100 (89.09)a	100 (89.09)a	100 (89.09)a
T_4	Thiamethoxam 25 WG	0.3 g/L	0.00 (0.91)	100 (89.09)a	100 (89.09)a	100 (89.09)a	100 (89.09)a	100 (89.09)a	100 (89.09)a
T_5	Acetamiprid 20 SP	0.2 g/L	0.00 (0.91)	33.33 (35.22)d	50.00 (45.00)b	66.67 (54.78)b	100 (89.09)a	100 (89.09)a	100 (89.09)a
T_6	*Beauveria bassiana*	5 gm/L	0.00 (0.91)	0.00 (0.91)e	0.00 (0.91)c	0.00 (0.91)d	3.33 (6.75)c	11.11 (16.17)c	61.31 (51.64)b
T_7	Neem oil 1500 ppm	5 ml/L	0.00 (0.91)	26.67 (31.00)d	50.00 (45.00)b	60.00 (50.77)c	64.07 (53.24)b	61.11 (51.49)b	56.55 (48.78)b
T_8	Pongamia soap	7.5 gm/L	0.00 (0.91)	83.33 (66.15)b	100 (89.09)a	100 (89.09)a	100 (89.09)a	100 (89.09)a	100 (89.09)a
T_9	Untreated (Control)	water	0.00 (0.91)	0.00 (0.91)e	0.00 (0.91)c	0.00 (0.91)d	6.67 (12.59)c	13.33 (21.15)c	23.33 (28.78)c
	S.Em ±		-	2.02	1.11	0.67	2.89	3.02	1.59
	C.D (0.05)		-	5.99	3.3	1.99	8.58	8.99	4.73
	CV (%)		-	7.44	3.22	1.89	7.41	7.57	3.74

Figures in parentheses are **arc sine** transformed with the following formulae: 1/4n for 0% and 100-1/4n for 100%.
Values followed by the same letter(s) do not differ significantly at P ~~p~~=0.05 (~~DMRT~~)

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Table 2. Effect of insecticides at their field recommended doses on mortality of European honey bee, *Apis mellifera L.* exposed 12 hours after treatment

Treatments		Dosage (ml or g/L)	Mean Corrected mortality (%)						
			0 h	2 h	4 h	6 h	12 h	24 h	48 h
T_1	Chlorpyriphos 20 EC	2.5 ml/L	0.00 (0.91)	63.33 (52.78)b	80.00 (63.93)b	82.59 (65.63)b	100.00 (89.09)a	100.00 (89.09)a	100.00 (89.09)a
T_2	Profenofos 50 EC	1 ml/L	0.00 (0.91)	16.67 (23.86)d	33.33 (35.22)d	61.85 (51.92)c	89.26 (70.87)b	92.13 (76.31)b	100.00 (89.09)a
T_3	Lambda cyhalothrin 5 EC	1 ml/L	0.00 (0.91)	6.67 (12.29)e	20.00 (26.57)e	24.07 (29.30)d	60.74 (51.23)c	100.00 (89.09)a	100.00 (89.09)a
T_4	Thiamethoxam 25 WG	0.3 g/L	0.00 (0.91)	96.67 (83.25)a	100.00 (89.09)a	100.00 (89.09)a	100.00 (89.09)a	100.00 (89.09)a	100.00 (89.09)a
T_5	Acetamiprid 20 SP	0.2 g/L	0.00 (0.91)	33.33 (35.22)c	50.00 (45.00)c	55.19 (47.99)c	85.93 (68.17)b	100.00 (89.09)a	100.00 (89.09)a
T_6	*Beauveria bassiana*	5 gm/L	0.00 (0.91)	0.00 (0.91)f	0.00 (0.91)f	17.41 (24.38)d	39.26 (38.77)d	36.11 (36.93)c	43.45 (41.22)b
T_7	Neem oil 1500 ppm	5 ml/L	0.00 (0.91)	16.67 (23.86)d	26.67 (31.00)de	27.41 (31.52)d	39.63 (38.94)d	51.85 (46.06)c	47.62 (43.63)b
T_8	Pongamia soap	7.5 gm/L	0.00 (0.91)	0.00 (0.91)f	0.00 (0.91)f	3.33 (6.75)e	3.33 (6.75)e	7.41 (9.98)e	42.26 (39.82)b
T_9	Untreated (Control)	water	0.00 (0.91)	0.00 (0.91)f	0.00 (0.91)f	3.33 (6.75)e	6.67 (12.59)e	16.67 (23.86)d	23.33 (28.78)c
	S.Em ±		-	3.24	1.74	3.37	3.15	3.84	3.22
	C.D (0.05)		-	9.64	5.16	10.03	9.37	11.4	9.55
	CV (%)		-	21.61	9.23	14.89	10.56	10.88	8.37

Figures in parentheses are **arc sine** transformed with the following formulae: 1/4n for 0% and 100-1/4n for 100%.
Values followed by the same letter(s) do not differ significantly at P ~~p~~=0.05 (~~DMRT~~)

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Table 3. Effect of insecticides at their field recommended doses on mortality of European honey bee, *Apis mellifera L.* exposed 24 hours after treatment

Treatments		Dosage (ml or g/L)	Mean Corrected mortality (%)						
			0 h	2 h	4 h	6 h	12 h	24 h	48 h
T_1	Chlorpyriphos 20 EC	2.5 ml/L	0.00 (0.91)	83.33 (66.15)a	86.67 (68.86)a	100.00 (89.09)a	100.00 (89.09)a	100.00 (89.09)a	100.00 (89.09)a
T_2	Profenofos 50 EC	1 ml/L	0.00 (0.91)	16.67 (23.86)b	33.33 (35.22)c	66.67 (54.74)c	68.06 (55.66)c	78.57 (62.60)c	91.07 (75.39)b
T_3	Lambda cyhalothrin 5 EC	1 ml/L	0.00 (0.91)	0.00 (0.91)d	6.67 (12.59)d	37.03 (37.45)d	76.39 (61.34)c	100.00 (89.09)a	100.00 (89.09)a
T_4	Thiamethoxam 25 WG	0.3 g/L	0.00 (0.91)	76.67 (61.22)a	86.67 (68.86)a	92.59 (76.72)b	100.00 (89.09)a	100.00 (89.09)a	100.00 (89.09)a
T_5	Acetamiprid 20 SP	0.2 g/L	0.00 (0.91)	20.00 (26.57)b	66.67 (54.99)b	74.07 (59.49)c	100.00 (89.09)a	100.00 (89.09)a	100.00 (89.09)a
T_6	*Beauveria bassiana*	5 gm/L	0.00 (0.91)	10.00 (15.30)bc	26.67 (31.00)c	22.22 (28.13)e	39.81 (38.94)d	39.29 (38.81)d	36.31 (36.99)cd
T_7	Neem oil 1500 ppm	5 ml/L	0.00 (0.91)	13.33 (21.15)b	23.33 (28.78)c	25.93 (30.51)de	27.78 (31.76)d	38.69 (38.36)d	45.24 (42.26)c
T_8	Pongamia soap	7.5 gm/L	0.00 (0.91)	3.33 (6.75)cd	63.33 (53.07)b	74.07 (59.49)c	87.96 (69.71)b	91.67 (75.89)b	91.07 (75.39)b
T_9	Untreated (Control)	water	0.00 (0.91)	0.00 (0.91)d	0.00 (0.91)e	10.00 (18.44)f	16.67 (23.86)e	23.33 (28.78)e	26.67 (31.00)d
	S.Em ±		-	3.63	3.50	2.58	2.47	2.79	3.45
	C.D (0.05)		-	10.78	10.39	7.67	7.33	8.27	10.26
	CV (%)		-	25.38	15.39	8.87	7.01	7.23	8.72

Figures in parentheses are **arc sine** transformed with the following formulae: 1/4n for 0% and 100-1/4n for 100%.
Values followed by the same letter(s) do not differ significantly at P ~~p~~=0.05 (~~DMRT~~)

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