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Monocrotophos induced changes in indole acetic acid production by *Staphylococcus aureus*

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ABSTRACT

Although extensive application of pesticides in crop fields has enhanced crop production, it could adversely affect the soil and the microbes inhabiting it. Several microbes are known to exhibit plant growth promoting traits like indole acetic acid production, siderophore production etc. The present study was designed to screen the bacteria inhabiting monocrotophos (Dimethyl (E)- 1 – methyl – 2 – (methyl – carbamoyl) vinyl phosphate) exposed sugarcane field soil. The dominant bacteria were Bacillus subtilis, Bacillus circulans, Bacillus firmns, Bacillus pumilus, Bacillus licheniformis, Bacillus cerens, Bacillus mycoides, Bacillus Paenibacillus, Pseudomonaspolymyca, Bacillus thuringiensis and Pseudomonasmacerans. Staphylococcus aureus was exposed to various concentration of monocrotophos (100, 200 and 300µl). Monocrotophos did not elicit any significant change in the Staphylococcus aureuspopulation. However, IAA production by Staphylococcus aureus significantly declined, which indicates that monocrotophos interferes with IAA metabolism.

Keywords: Indole acetic acid (AA), Monocrotophos, *Staphylococcus aureus*, Total Heterotrophic Bacteria (THB).

INTRODUCTION

Pesticides applied in agricultural lands to control pest accumulate their residues and metabolites in soil. Resultantly, such agrochemicals beyond certain levels pose serious threat to both the rhizospheric organisms and associated biotic processes which are governed by the rate of application, the toxicity and activity spectrum of pesticides and the persistence and availability of chemicals in soils [1]. Indole acetic acid (IAA) is a common natural auxin and is a product of L-tryptophan metabolism in microorganisms. Approximately 80% of rhizospherebacteriacan secrete IAA[2].*Streptomyces* spp., inhabiting the rhizospheres of various plants, also serves as good source of IAA. Several *Streptomyces* species, such as *S. olivaceoviridis, S. rimosus,S. rochei*and *Streptomyces* spp. from the tomato rhizosphere, have the ability toproduce IAA and improve plant growth by increased seed germination, root elongation and root dry weight [3,4,5]. In this context, many pesticides are known to produce deleterious effects on the populations and activity of beneficial soil microorganisms that catalyze various biological processes important to soil fertility and plant growth [6]. With this view, the present study was initiated to identify the dominant bacteria prevalent in monocrotophos applied sugarcane field soil. Further, to determine the impact of monocrotophos on the growth of dominant bacteria *in vitro* condition. In addition to evaluate the impact of monocrotophos on production of IAA by dominant bacteria.

MATERIALS AND METHODS

Collection of soil samples: Soil samples were collected from sugarcane cultivated land, (Naganoor) Thogamalai, Karur in Triplicate (10g soils) in sterile bottles from five different places. Soil samples were thoroughly mixed and a 10g of the mixture was homogenized using a glass rod.

Dynamics of *Staphylococcus aureus* population in soil samples exposed to monocrotophos: In order to identify Total Heterotrophic Bacteria (THB), *Aeromonas*sp., *Actinomycetes* sp., and *Staphylococcus* sp.,a serial dilution

assay was carried out in 0.9% NaCl solution and 10µl of diluted suspension was spread plated on nutrient agar, *Aeromonas*agar base, *Actinomycetes*agar base and *Staphylococcus* Vogel-Johnson agar base medium, respectively. The plates were incubated at 28 ± 2 ^{0}C for 24 hours. Bacterial isolates were identified by the methods mentioned in Bergeys manual of Determinative bacteriology [7]. *Staphylococcus aureus* was chosen for further studies.

Exposure of the soil samples to pesticide: The soil samples were exposed to 200µl, 400µl, 600µl of monocrotophos in minimal salt medium in triplicates and were incubated with 1 ml of *Staphylococcus aureus*culture (3.4533E2 ± 52.66983 cfu/ml). A control was maintained simultaneously and experiment was carried out for a period of 7 days. In order to study the growth pattern of *Staphylococcus aureus*, optical density value was recorded on the 1st and 7th day of incubation. Further, the colony forming units (cfu) were enumerated to test the viability of bacteria.

Quantitative assay of IAA: Indole-3- acetic acid synthesized by bacterial strains was quantitatively evaluated by the method of Gordon and Weber [8] and later modified by Brick *et al.*,[9]. Selected bacterial strains were grown in Luria Bertani (LB) broth. Luria Bertani broth (100 ml) having fixed concentration of tryptophan (100 mg/ml)and supplemented with 0, 200, 400 and 600µl of recommended rate of each pesticide was inoculated with 1 mL culture of *Staphylococcus aureus* bacterial isolates (34.0000 ± 0.57735×10^{10} cfu/ml) and was incubated for seven days at 28 ± 2 ^{0}C with shaking at 125 rpm. After seven days, 5 ml of culture of each treatment was centrifuged (9,000 rpm) for 15 minutes. and an aliquot of 2 ml supernatant was mixed with 100 µl of orthophosphoric acid and 4 ml of salkowsky reagent (2% 0.5 M $FeCl_3$ in 35% per-chloric acid) and incubated at 28 ± 2 ^{0}C in darkness for 1 hour. The absorbance of developed pink colour was read at 530 nm. IAA concentration in the supernatant was determined using a calibration curve of pure IAA as a standard. Simultaneously at the end the 7thday, Total Heterotrophic Bacteria (THB) were enumerated.

RESULTS AND DISCUSSION

Total Heterotrophic Bacteria (THB), *Aeromonas*, *Actinomycetes* and *Staphylococcus aureus* population were assessed. Total Heterotrophic bacteria (THB) registered 1.4060E10 ± 9.02757E9cfu/ 10µl. *Aeromonas*, *Actinomycetes* and *Staphylococcus* population in the sugarcane crop field soil were 2.8000E10 ± 1.45717E10cfu / 10µl; 2.3000E10 ± 7.63763E9cfu/ 10µl and 4.6667E10 ± 4.48454E9cfu/ 10µl, respectively (Table 1).

Table 1: Bacterial populationinsugarcanecrop field soil.

Bacteria	Bacterial population cfu/ 10µl
THB	1.4060E10 ± 9.02757E9 a
AeromonasSp.	2.8000E10 ± 1.45717E10 a
ActinomycetesSp.	2.3000E10 ± 7.63763E9 a
Staphylococcus Sp.	4.6667E10 ± 4.48454E9 a
F	2.033^{NS}
Significance	0.188

Not significant at P < 0.05; n = 3, Values are Mean ± Standard Error, E = Exponent, NS = Not significant
cfu = Colony forming units

In a column, figures having dissimilar letters differ significantly according to Duncan New Multiple Range Test (DMRT)

Dominant bacteria observed in the present study were *Bacillus subtilis, Bacillus circulans, Bacillus firmns, Bacillus pumilus, Bacillus licheniformis, Bacillus cerens, Bacillus mycoides, Bacillus Paenibacillus, Pseudomonas polymyca, Bacillus thuringiensis and Pseudomonasmacerans* (Table 1a).

Table 1a:Monocrotophos resistant bacteria isolated from sugarcane crop field soil.

Bacteria
Bacillus subtilis
Bacillus circulans
Bacillus firmns
Bacillus pumilus
Bacillus licheniformis
Bacillus cerens
Bacillus mycoides
Bacillus Paenibacillus
Pseudomonaspolymyca
Bacillus thuringiensis
Pseudomonasmacerans

Impact of monocrotophos on *Staphylococcus aureus*: *Staphylococcus aureus* was selected in the present study to evaluate the pesticide stress on bacteria. *Staphylococcus aureus* were exposed to 200µl, 400µl, 600µl of monocrotophos for a period of seven days. A control group was maintained simultaneously. Initial and final O.D value were recorded. It is observed form the present result that after 24 hours, significantly higher (F = 15.608, P < 0.001) O.D was registered in 200µl (0.3433 ± 0.02728) of monocrotophos, when compared with control: (0.2900 ± 0.01732); 600µl: (0.2433 ± 0.01453) and 400µl: (0.1733 ± 0.00882) (Table 2). At the end of the 7th day, significantly higher O.D (F= 11.268, P < 0.01) was recorded in the control group (0.1767 ± 0.00882) when compared with 200µl, 600µl and 400µl Monocrotophos(0.1333 ± 0.01856; 0.1133 ± 0.00882 and 0.0867 ± 0.00333, respectively) (Table 2a).

Table 2: Variation in the O.D value after 24 hours of exposure of *Staphylococcusaureus* tomonocrotophos.

Treatment	Optical Density
Control	0.2900 ± 0.01732 ab
200 µl	0.3433 ± 0.02728 a
400 µl	0.1733 ± 0.00882 c
600 µl	0.2433 ± 0.01453 b
F	15.608 ***
Significance	0.001

**** Significant at P < 0.001*
n = 3, Values are Mean ± Standard Error

In a column, figures having dissimilar letters differ significantly according to Duncan New Multiple Range Test (DMRT)

Table 2a: Variation in the O.D value after 7 days exposure of *Staphylococcus aureus*to monocrotophos.

Treatment	Optical Density
Control	0.1767 ± 0.00882a
200 µl	0.1333 ± 0.01856 b
400 µl	0.0867 ± 0.00333 c
600 µl	0.1133 ± 0.00882bc
F	11.268 **
Significance	0.003

*** Significant at P < 0.01*
n = 3, Values are Mean ± Standard Error

In a column, figures having dissimilar letters differ significantly according to Duncan New Multiple Range Test (DMRT)

Indole acetic acid production by monocrotophos exposed *Staphylococcus aureus*: The result presented (Table 3) reveal that significant (F = 60.778, P < 0.001) decline in IAA production by *Staphylococcus aureus* was observed after exposure to monocrotophos. Furthermore, dose – dependent relationship between monocrotophos concentration and IAA production was observed. As the concentration of monocrotophos increased, the concentration of IAA decreased. (100µl: 29.3333 ± 0.88192 µl/ ml; 200µl: 22.3333 ± 1.76363 µl/ ml; 300µl: 12.6667 ± 1.20185 µl/ ml). Control registered IAA production of 34.0000 ± 0.57735 µl/ml. Further, simultaneously along with estimation of IAA production, *Staphylococcus aureus* population was assessed. No significant variation in *Staphylococcus aureus* population was evinced after exposure to monocrotophos for a period of 7 days (control: 1.2967E11 ± 1.06823E10cfu/10µl; 100µl: 2.9767E11 ± 2.96273E9cfu/10µl; 200µl: 8.7100E10 ± 3.92900E10cfu/10µl; 300µl; 2.3482E11 ± 1.16350E11cfu/10µl) (Table 4).

Table 3: Impact of monocrotophoson IAA production by *Staphylococcus aureus.*

Treatment	IAA Production (µl/ml)
Control	34.0000 ± 0.57735 a
100 µl	29.3333 ± 0.88192 b
200 µl	22.3333 ± 1.76363 c
300 µl	12.6667 ± 1.20185 d
F	60.778****
Significance	0.001

***** Significant at P < 0.001; n = 3, Values are Mean ± Standard Error*
IAA = Indole acetic acid

In a column, figures having dissimilar letter differs significantly according to Duncan New Multiple Range Test (DMRT)

Table 4: *Staphylococcus aureus*populationon the 7 days of monocrotophos exposure.

Treatment	*Staphylococcus aureus*(cfu/ 10µl)
Control	1.2967E11 ± 1.06823E10 a
100 µl	2.9767E11 ± 2.96273E9 a
200 µl	8.7100E10 ± 3.92900E10 a
300 µl	2.3482E11 ± 1.16350E11 a
F	2.438^{NS}
Significance	0.139

Not significant at P < 0.05 ; n = 3, Values are Mean ±Standard Error, E = Exponent
cfu = Colony forming units

In a column, figures having dissimilar letters differ significantly according to Duncan New Multiple Range Test (DMRT)

Soil used for the present study was exposed to MCP for several years. Hence, the organisms isolated were those that could survive in the soil either due to tolerance to the pesticide or due to their ability to degrade it. Monocrotophos did not induce any change in *Staphylococcus aureus* population in sugarcane field soil. This observation is contradictory with the findings of Umamaheswari and Murali [10], who have reported that monocrotophos reduces the bacterial population of sugarcane crop field soil. Further, they have also observed monocrotophos resistant bacteria in sugarcane crop field soil and have demonstrated that monocrotophos resistant trait in bacteria was plasmid borne. The present findings also disagrees with that of Umamaheswari *et al.*,[11] who have observed significant decline in Total Heterotrophic Bacteria (THB) of chilly field soil exposed to endosulfan.

Though *Staphylococcus aureus* is a pathogenic organism, it has also the ability to produce IAA and siderophore, which are important component in plant growth enhancement. From this study, it is evident that monocrotophos interferes with the IAA metabolism, which is reflected in the reduction of production of IAA by *Staphylococcus aureus* on exposure to monocrotophos.

Among the phytohormones, IAA and its analogues, synthesized from tryptophan, are the main auxin produced in most plants, controlling many important physiological processes including cell enlargement and division, tissue differentiation, root initiation, root growth inhibition, increased growth rate, phototropism, geotropism and apical dominance [12]. In this study, the *Staphylococcus aureus* produced a substantial amount of IAA.

Quantitative analyses of IAA in a submerged culture indicated that *S. purpurascens*, *S. coelicolor*, *S. olivaceus*and *S. kasugaensis* produce significant levels (>5 mg/ml)[13,14], of IAA. Some plant-growth-promoting rhizobacteria have been found to stimulate root proliferation byIAA biosynthesis [15]. It is therefore possible that IAA couldact as a reciprocal signalling substance [16] in *Streptomyces*-plant interactions.

The results of the present investigation reflect that *Staphylococcus aureus* population was not affected by monocrotophos. But, IAA production by *Staphylococcus aureus* declined on exposure to monocrotophos and was found to be concentration dependent. These observations indicate that monocrotophos interferes with the IAA metabolism. This could in turn affect plant growth.

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