Effects of Acetamiprid, Carbofuran on Soil Enzyme Activities in Groundnut (*Arachis hypogaea* L.) Soils.

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Abstract— The effects of two pesticides viz., acetamiprid (Neonicotinoid) and carbofuran (Carbamate) were evaluated on amylase and cellulase enzyme activities in the groundnut cultivated soils of Anantapur District. Compared to untreated control, application of both acetamiprid and carbofuran resulted in the stimulation of amylase and cellulase enzyme activities at lower concentrations (10.0 kg $ha^{-1}$to 5.0 kg ha^{-1}) but inhibition at higher concentration (7.5 kg $ha^{-1}$to 10.0 kg ha^{-1}) over untreated control after 10 days of incubation. However the stimulatory effect was continued up to 20 days of incubation in both soil samples. Whereas, the decline phase was started after 20 days and the minimum enzyme activities were noticed at the end of 40 days of incubation. But higher concentrations of insecticides at the level of 7.5 to 10.0 kg ha^{-1} were either toxic or innocuous to amylase and cellulase activities in both soil samples.

Index Terms— Acetamiprid, Amylase, Carbofuran, Cellulase, Groundnut (*Arachishypogaea* L.),

1 INTRODUCTION

Under normal agricultural practices tremendous uses of agrochemicals are there in each year to boost crop production. The use of chemical pesticides in Indian agriculture drastically increased in recent years. The word pesticides include a heterogeneous group of chemicals developed to control a variety of pests; pesticides are generally categorized as insecticides, herbicides and fungicides according to the type of pest which they have shown efficacious action [1]. Anantapur, a semi arid region of Andhra Pradesh, India. Although ranks first in area of groundnut (*Arachishypogaea* L.) cultivation in the state [2], its productivity is low fluctulating around 9q/ha on average. One of the main reasons attributed for such situation is insect pest pest problem, abiotic factors etc., [3] hence pesticides particularly acetamiprid and carbofuran has become an indispensable tool in agriculture to combat various pests on groundnut [4]; [5]). The neonicotinoid insecticide acetamiprid (N-[6-Chloro-3-Pyridyl)Methyl]-N-Cyano-N-Methyl-Acetamidine is a new generation insecticide with ground and aerial application against aphids, leafhoppers, whiteflies, thrips etc., acetamiprid poses low risks to the environment relative to most other insecticides and its use would pose minimal risk to non target plants [6]. The Carbofuran (2,3-dihydro-2,2-dimethyl-7-benzo-furanyl methyl carbamate) is extensively used as a soil incorporated N- methylcarbamate insecticide, nematicide to control a variety of insect pests [7]. These agrochemicals are applied either directly to the soil or transported from the treated crops, but they are imposing a treat to the soil environment [8] killing the non-target beneficial microorganisms that are responsible for enhancement of soil fertility [9].

Soil is a living-dynamic, non-renewable resource and its conditions influence food production, environmental efficiency and global balance [10]; [11]). The quality of soil depends in part on its natural composition, and also on the changes caused by human use and management [12]). Human factors influencing the environment of the soil can be divided into two categories: those resulting in soil pollution and those devoted to improve the productivity of soil [13]. A soil is biologically active, when biological processes proceed rapidly, i.e. in a distinct span of time a lot of metabolites are produced [14]). A variety of methods were developed to measure soil biological activity. All these methods are not suited to produce generally accepted results, but they give relative information about the ecological status of soil ecosystem [15]; [16]). The soil enzymatic activity assay is only one way to measure the ecosystem status of soils.

Soil quality and its degradation depend on a large number of physical, chemical, biological, microbiological and biochemical properties, the last two being the most sensitive since, they respond rapidly to changes. The microbiological activity of a soil directly influences ecosystem stability and fertility and it is widely accepted that a good level of microbiological activity is essential for maintaining soil quality. The soil microbiological activity viz., the enzymatic activities play a key role in soil nutrient cycling, its activity is essential in both the mineralization and transformation of organic matters and plant nutrients in soil ecosystem. Soil enzyme activities are very sensitive to both natural and anthropogenic disturbances and show a quick response to the induced changes. Therefore, enzyme activities can be considered effective indicators of soil quality changes resulting from environmental stress or management practices. These soil enzymes play a fundamental role in establishing biogeochemical cycles and facilitate the development of plant cover. It is an important aspect of the below-ground processes and give insight into the relative changes in below-ground system functioning as a plant community develops over time.

The effect of pesticides on soil microorganism can be

assessed following two ways a) directly by estimating the soil microbial population and biomass and b) indirectly by studying the soil metabolism through soil respiration and soil enzyme activities Microbial respiration and enzymatic activities were used as appropriate indicators for highliting the impact of land use management, soil quality monitoring and pollution [17] some of the most studied enzymes in soil are amylase and cellulose because of the active involvement in soil carbon cycle. Amylase plays an important role in biochemical reactions and nutrient cycling. Cellulase can catalyze hydrolysis of 1, 4, beta- D-glycosidic bonds of cellulose and is also an important indicator for carbon circulation. Apparently, it has become necessary to determine the effects of agronomically needed pesticides (Acetamiprid and Carbofuran), applied at recommended levels and at higher doses, in order to establish the significance, in terms of biogeochemical reactions and nutrient cycling.

Several studies were conducted to find out the effects of pesticides on soil enzymes [18], [19] most of these studies conduct the pesticides at higher doses inhibit enzymatic activities. However few studies were conducted on acetamiprid and carbofuran which showed no concrete conclusion.

Hence the present study was carried out to determine the influence of insecticides on the activity of cellulase and amylase in two groundnut soils of Anantapur district Andhra Pradesh, India.

2 Materials and Methods

2.1 Soils

Soil samples, black and red clay with previous pesticide history were collected from groundnut-cultivated fields of Anantapur district, Andhra Pradesh, India, were chosen with a known history of pesticides use, from a depth of 12 -15cm, air-dried and sieved through 2 mm sieve before usage. Mineral matter of soil samples such as sand, silt, and clay contents were analyzed with use of different sizes of sieves by following the method of Alexander (1961) [20]. Sent percent water-holding capacity of soil samples was measured by finding amount of distilled water added to both the soil samples to get saturation point and then 60% water holding-capacity of soil was calculated Jhonson and Ulrich method (1960) [21]. Soil pH was measured at 1:1.25 soil to water ratio in systronics digital pH meter with calomel glass electrode assembly. Organic carbon content in soil samples was estimated by Walkley- Black method, and the organic matter was calculated by multiplying the values with 1.72 [22]. Electrical conductivity of soil samples after addition of 100 ml distilled water to 1 gram soil samples was measured by a conductivity bridge. Total nitrogen content in soil samples was determined by the method of Micro-kjeldhal method [22]. Content of inorganic ammonium-nitrogen in soil samples after extraction of 1M KCl by Nesslerization method [22], contents of nitrite-nitrogen [23] and contents of nitrate- nitrogen by Brucine method [24] after extraction with water were determined respectively. Physicochemical characteristics of the two soils were listed in Table1.

2.2 Insecticides

In order to determine the influence of selected insecticides on the microbial activities, commercial grades of acetamiprid and carbofuran were obtained from Daulatabad Road, Gurgaon-122001, Haryana and Vantech Chemicals Ltd.Sy. No. 806&180/7, Khazipally, Jinnaram Mandal, Medak Dist., (A.P.).

2.3 Soil treatment

The soil ecosystem stimulating non-flooded portions of the soil samples were added in test tubes (25 x 150 mm) and moistened with water in order to maintain at 60% water- holding capacity. Same model was used previously to elucidate the effect of insecticides on microbial activities by Gooty Jaffer Mohiddin et al. (2013) [25].

2.4 Cellulase (EC 3.2.1.4) and Amylase (EC 3.2.1.1)

Five gram portions of the soil samples were weighed and dispersed into sterile test tubes (25 x 150 mm). Selected insecticides from stock solutions were added at the levels of 10, 25, 50, 75 and 100 μg g^{-1} soil, which were equivalent to field application rates of 1.0, 2.5, 5.0, 7.5 and 10.0 kg ha^{-1} respectively. Soil samples were mixed thoroughly for uniform distribution of added insecticides. Soil samples without insecticide treatment served as controls. Three triplicates were maintained for each treatment at room temperature (28 ± 4°C) with 60% water-holding capacity throughout the incubation period. After desired intervals of incubation, soil samples were extracted in distilled water for estimation of enzyme activities. Similar model was used earlier by Singaram and Kamalakumari [26]; Jaffer Mohiddin et al. [25].

In order to determine cellulase enzyme activity in soils, the method employed for the assay of cellulase was developed by Cole [27] and followed by Tu [28] ;[29]). After 15 minutes, 10 ml of 1% carboxy methyl cellulose (CMC) was added as a substrate, followed by10 ml of acetate buffer (pH 5.9) and incubated for 24 hours to determine the reducing sugar content in the filtrate [30]. In another experiment, cellulase activity was determined at 10, 20, 30 and 40 days of soil incubation with pesticides. Testing samples were passed through Wattman No. 1 filter paper and the filtrate was assayed for the amount of glucose by Nelson method [31] in a Spectronic 20 D spectrophotometer.

The method employed for the assay of amylase was developed by Cole [27] and followed by Tu [28], [29]. The soil

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samples were transferred to 100 ml Erlenmeyer flasks and were treated with 1 ml of toluene to arrest the enzyme activity. After 15 minutes, 6ml of 0.2M of acetate phosphate buffer (5.5 pH) containing 2% starch was added to each of the testing samples and closed with cotton plugs. After 24 hours and 72 hours of incubation, the testing samples were made up to a volume of 50ml with sterile distilled water and passed through Wattman No. 1 filter paper and the filtrate was assayed for the amount of glucose by Nelson's method [31] in a Spectronic 20 D spectrophotometer.

3 Statistical analysis

The concentration of the cellulase, invertase and amylase was calculated based on soil weight (oven dried). Data were analyzed using one-way ANOVA, and the differences contrasted using Duncan's multiple range test (DMRT) (Gooty Jaffer Mohiddin et al. [25]. All statistical analysis was performed at $P \leq 0.05$ using SPSS statistical software package.

4 Results and discussion

The black and red clay soils were predominantly used for the cultivation of groundnut (*Arachis hypogaea* L.) in the Anantapur district of Andhra Pradesh, India. Hence, these soils were selected to study the effect of insecticides on enzyme activities. In general, the organic matter content is high in black soil [25]. Therefore, the biological activity was also pronounced more in black soil than in red soil under the influence of insecticides. There have been many reports on the effects of pesticide on soil enzyme activities [32]; [33]; [34]) and it has been observed that the responses of soil enzymes to different pesticide are not the same.

Since the enzyme activity has been considered as a very sensitive indicator, any disturbance due to biotic or environmental stresses in the soil ecosystem may affect soil biological properties. Our analysis revealed that cellulase activity was significantly increased from 2.5 to 5.0 kg ha^{-1}, where as the activity was decreased at higher concentrations (7.5 to 10.0 kg ha^{-1}) of pesticides in both soils (Table 2). The cellulase activity was significantly enhanced at 5.0 kg ha^{-1} level in both soils. The insecticides, acetamiprid and carbofuran showed individual increments in cellulase activity ranged from a low increase, 38– 56%, 32 – 67% and 24 – 55%, 4 – 15% in comparison to control (Table 2). The stimulatory concentration (5.0 kg ha^{-1}) induces the highest cellulase activity after 20, 30 and 40 days of incubation in black clay soils (Fig 2a) with acetamiprid and carbofuran when compared to control. Where as in red clay soil a similar trend was followed by acetamiprid and carbofuran, which induces the highest cellulase activity after 20, 30 and 40 days of incubation (Fig 2b). The relatively low activity of cellulase might result from the toxic effect of acetamiprid and carbofuran on soil microorganisms, which in turn produces cellulase. The inhibition of cellulase activity by acetamiprid and carbofuran could be attributed to the properties of acetamiprid and carbofuran . Similar type of reports were identified by Ramudu et al. [35]; Jaffer Mohiddin et al. [36], [25], with imidacloprid, acephate and flubendiamide, spinosad. Similar observations were made by Katayama and Kuwatsuka [37] and Jayamadhuri [38] on the cellulase activity. Analogous report was obtained by Ismail et al. [39]; [40] on application of metolachor to Malaysian soil. Gigliotti et al. [41] also reported that bensulfurn methyl at 16 and 160 mug/g inhibited cellulase activity in soil samples. In a diverse study made by Gherbawy and Abdelzaher [42], alteration in the activity of cellulase by metalaxyl was marked in pure fungal cultures. Similar results were obtained by Arinze and Yubedee [43] that kelthane and fenvalerate caused inhibition to enzyme activity.

Amylase activity showed a variable pattern in response to different insecticide concentrations after 10 days of incubation (Table 3a and 3b). Amylase activity increased under lower doses and decreased under higher doses in comparison to controls in black and red clay soils. Flubendiamide and spinosad significantly enhanced maximum enzyme activity at 2.5 kg ha^{-1}. Amylase activity showed an individual increment of 28-78%, 85-127%, 39-94%, 23-48%, in black clay soil and 3-10%, 9-24%, 6-15%, 1-4%, in comparison to control at 24 hours and 72 hours received 2.5 kg ha^{-1} respectively in red clay soil. Our results were in contrast with the several reports [44]; [36]; [28];[29]; [45]; [46]), triazophos, a phosphorothioate triazole is stimulated for amylase at 5 and 10 mg/kg incubated for three days in an organic soil. As per the observation made by the Prasad and Mathur [47] the amylase activity increased during germination in both control, and Cuman treated seeds at 0.25, 0.5, 0.75 and 1% respectively. Interaction effects on soil enzyme activities, including amylase activity received least attention. There were only a few isolated reports on interaction effects between two chemical compounds in axenic culture studies with algae, cyanobacteria and fungi [48]; [49], [50]. Kennedy and Arathan [51] reported that application of carbofuran at 1 and 1.5 kg ha^{-1} significantly reduced the activity of soil enzymes, viz., alpha -amylase, beta -glucosidase, cellulase, urease and phosphatase up to 30 days after carbofuran application. However, application of carbofuran at the recommended level (0.5 kg a.i. ha^{-1}) had no significant effect upon the activity of soil enzymes, which are biologically significant as they play an important role not only in the soil chemical and biological properties but also affect the nutrient availability to plants. Rate of amylase activity followed the same trend of initial stimulation followed by inhibition as reported by Rangaswamy and Venkateswarlu [52]) and Vijay Gundi et al. [9].

5 Conclusions

Results from this study indicated that the cellulase enzyme activity was profoundly increased upto 5.0 kg ha^{-1} whereas at higher concentrations (7.5 to 10.0 kg ha^{-1}) of pesticides, the enzyme activity was dramatically decreased in both soils. Amylase enzyme activity was dramatically enhanced upto 5.0 kg ha^{-1}, whereas further increase in the pesticide concentration repression in the enzyme activity was noticed in both soils. Overall soil enzymes were affected by the application of acetamiprid and carbofuran at higher concentrations

(7.5 - 10.0kg ha^{-1}). However, as an important agent for the control of plant pathogens, acetamiprid and carbofuran is often used at much higher than the recommended dosage.

On the whole, acetamiprid and carbofuran at a normal field doses (1.0 – 5.0kg ha^{-1}) would not pose a threat to soil enzymes among them acetamiprid is more effective than carbofuran in inducing the cellulase and with exception of amylase enzyme activity at normal field rates (1.0-5.0kg ha^{-1}). However, when acetamiprid and carbofuran concentration increased (7.5-10.0kg ha^{-1}), the threat to soil, cellulase and amylase was increased.

6 HELPFUL HINTS

6.1 Figures and Tables

Table 1 Physicochemical properties of the soils

Properties	Black clay soil	Red clay soil
Sand (%)	68.45	53.25
Silt (%)	21.45	27.12
Clay (%)	10.0	19.8
pH [a]	7.8	6.7
Water holding capacity (ml g^{-1} soil)	0.7	0.4
Electricalconductivity (m.mhos)	258	232
Organic matter [b] (%)	1.34	0.74
Total nitrogen [c] (%)	0.086	0.038
NH_4^+ - N (µg g^{-1} soil)[d]	6.96	6.01
NO_2^- - N (µg g^{-1} soil)[e]	0.58	0.42
NO_3^- - N (µg g^{-1} soil)[f]	0.94	0.73

Where, a = 1:1.25 = Soil: Water slurry,
b = Walkley-Black method (Johnson and Ulrich, 1960),
c = Micro-Kjeldhal method (Johnson and Ulrich, 1960),
d = Nesslerization method (Johnson and Ulrich, 1960),
e = Diazotization method (Ranney and Bartlett, 1972),
f = Brucine method (Barnes and Folkard, 1951)

Table 2. Influence of selected insecticides on activity of cellulase* in black and red soil after 10 days.

Insecticide concentration (kg ha^{-1})	Black soil		Red soil	
	Acetamiprid	Carbofuran	Acetamiprid	Carbofuran
0.0	2090±1.154 e (100)	2090±1.154 f (100)	1680±2.57 d (100)	1680±2.22 d (100)
1.0	2568±1.732 c (123)	2220±0.577 d (106)	1869±2.43 c (111)	1689±5.2 c (101)
2.5	2858±0.577 b (138)	2758±2.309 c (132)	2078±1.36 b (124)	1756±3.13 b (104)
5.0	3258±1.732 a (156)	3485±3.464 a (167)	2599±1.19 a (155)	1926±3.12 a (115)
7.5	2180±2.309 d (104)	2780±5.773 b (133)	1078±1.06 e (64)	1588±5.24 e (94)
10.0	1580±2.309 f (75)	2215±2.886 e (106)	998±0.66 f (59)	1021±5.92 f (61)

*µg glucose per gram soil formed after 24 hours of incubation with 1% carboxy methyl cellulose (CMC).Each column is mean ± S.E. for six concentrations in each group; columns not sharing a common letter (a, b, c, d, e and f) differ significantly with each other ($P \leq 0.05$; DMRT).

Table 3a. Influence of selected insecticides on activity of amylase* in black soil incubated for 24 and 72 hours after 10 days.

Insecticide concentration (kg ha^{-1})	Black Soil			
	Acetamiprid		Carbofuran	
	24 hrs	72 hrs	24 hrs	72 hrs
0.0	180±4.35 f (100)	260±1.06 f (100)	180±0.72 f (100)	260±2.22 e (100)
1.0	410±2.57 c (228)	480±1.96 c (185)	250±0.66 c (139)	320±0.53 c (123)
2.5	500±2.43 a (278)	590±2.83 a (227)	350±1.8 a (194)	385±0.61 a (148)
5.0	450±2.12 b (250)	520±1.8 b (200)	300±2.22 b (167)	340±2.06 b (131)
7.5	370±1.36 d (205)	390±0.52 d (150)	230±3.82 d (128)	278±1.25 d (107)
10.0	250±1.19 e (139)	280±0.62 e (108)	200±3.6 e (111)	240±1.13 f (92)

*µg glucose per gram soil formed after 24 and 72 hours of incubation with 2% starch.
Each column is mean ± S.E. for six concentrations in each group; columns not sharing a common letter (a, b, c, d and e)

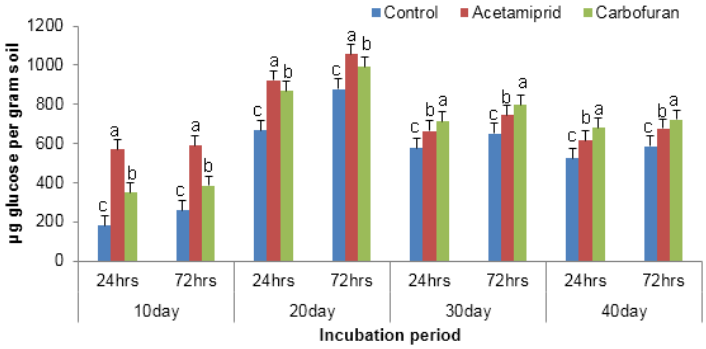
differ significantly with each other ($P \leq 0.05$; DMRT).

Table 3b. Influence of selected insecticides on activity of amylase* in red soil incubated for 24 and 72 hours after 10 days.

Insecticide concentration (kg ha^{-1})	Red Soil			
	Acetamiprid		Carbofuran	
	24 hrs	72 hrs	24 hrs	72 hrs
0.0	310±3.2 c (100)	380±1.56 d (100)	310±1.61 c (100)	380±1.61 c (100)
1.0	320±2.13 b (103)	396±1.25 b (105)	330±2.4 c (106)	345±2.4 d (101)
2.5	340±3.13 a (110)	415±1.13 a (124)	350±2.13 b (115)	396±2.13 a (104)
5.0	310±2.06 c (100)	386±3.2 c (104)	310±2.52 a (100)	345±2.4 d (94)
7.5	220±1.98 d (71)	246±2.13 e (65)	250±3.13 e (81)	298±2.52 e (78)
10.0	100±1.72 e (32)	228±0.53 f (60)	220±1.79 f (71)	258±2.13 f (68)

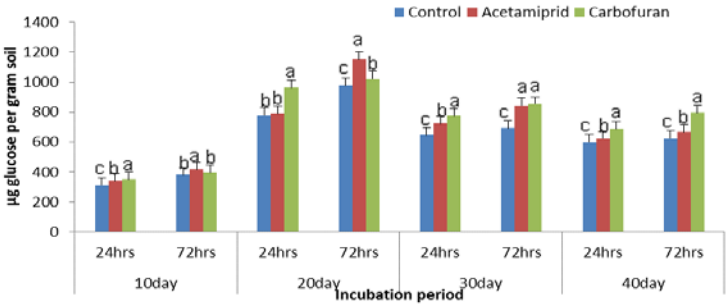
*µg glucose per gram soil formed after 24 and 72 hours of incubation with 2% starch.
Each column is mean ± S.E. for six concentrations in each group; columns not sharing a common letter (a, b, c, d and e) differ significantly with each other ($P \leq 0.05$; DMRT).

Fig 1a: Influence of acetamiprid and carbofuran on cellulase*activity in black clay soil at 5.0 kg ha^{-1}



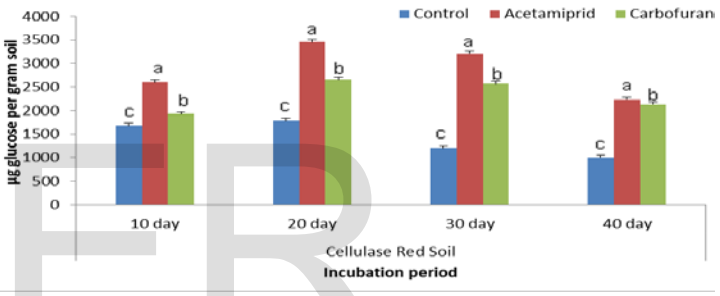
*µg glucose per gram soil formed after 24 hours incubation with Carboxy methyl cellulose (CMC).
The values are the means ± S.E. for each incubation period, are not significantly different ($P \leq 0.05$) from each other ac cording to Duncan's multiple range (DMR) test.

Fig 1b: Influence of acetamiprid and carbofuran on cellulase*activity in red clay soil at 5.0 kg ha^{-1}



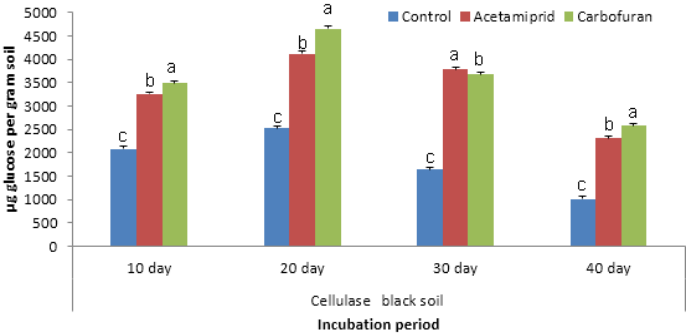
*µg glucose per gram soil formed after 24 hours incubation with Carboxy methyl cellulose (CMC). The values are the means ± S.E. for each incubation period, are not significantly different ($P \leq 0.05$) from each other ac cording to Duncan's multiple range (DMR) test.

Fig 2a: Influence of acetamiprid and carbofuran on amylase*activity in red clay soil at 2.5 kg ha^{-1}



*µg glucose per gram soil formed after 24 hours incubation with Carboxy methyl cellulose (CMC).
The values are the means ± S.E. for each incubation period, are not significantly different ($P \leq 0.05$) from each other ac cording to Duncan's multiple range (DMR) test.

Fig 2b: Influence of acetamiprid and carbofuran on amylase* activity in red clay soil at 2.5 kg ha^{-1}



*µg glucose per gram soil formed after 24 hours incubation with Carboxy methyl cellulose (CMC).
The values are the means ± S.E. for each incubation period, are not significantly different ($P \leq 0.05$) from each other ac cording to Duncan's multiple range (DMR) test.

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