4. RESULTS AND DISCUSSION
The effect of neem cake-amendment on population of total bacteria, nitrifiers, Azospirillum sp., and fungi was determined in an alluvial soil planted to mulberry. Microbial activities related to nitrogen transformations in soil such as ammonification and nitrification were also chosen to determine the impact of neem cake applications. Furthermore, the changes, if any, in the release of extracellular enzymes into the soil by different microorganisms were investigated. In each case, the influence of single (at 0-day) and two applications (at 0-day and after 90 days of plant growth) of neem cake at the rate of 100kg/acre was studied. Initially, some of the morphological growth parameters of the potted plants were recorded before collecting the soil for microbiological assays.

4.1. Effect of neem cake-amendment on plant growth

The data on plant height, leaf number and leaf area of mulberry in response to single or two applications of neem cake are presented in Table 3. Compared to untreated control plants, no change in height was observed in plants which received single soil-application of neem cake. Increased age of the plant greatly increased the height of the potted plants raised in unamended and neem cake-amended soil samples.
Table 3. Height (cm), leaf number, and leaf area (cm$^2$) of mulberry plants raised in unamended and neem cake-amended [single (0-day) and two applications (0 and 90 days)] soil.

<table>
<thead>
<tr>
<th>Morphological parameter</th>
<th>90th day after single application</th>
<th>90th day after two applications</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unamended</td>
<td>Amended</td>
</tr>
<tr>
<td>Plant height (cm)</td>
<td>18a</td>
<td>23a</td>
</tr>
<tr>
<td>Leaf number</td>
<td>20a</td>
<td>24b</td>
</tr>
<tr>
<td>Leaf area (cm$^2$)</td>
<td>48a</td>
<td>65b</td>
</tr>
</tbody>
</table>

The mean values (n = 3), in each row, followed by the same letter are not significantly different (P ≤ 0.05) from each other according to Duncan's new multiple range (DMR) test.
There was a significant increase in leaf number in plants from amended soil samples compared to control plants. The plants in neem cake-amended pots, that received two applications showed greater number of leaves. The leaf area was in direct relation to the leaf number in case of plants grown in unamended and neem cake-amended soil.

The present observation is thus in clear conformity with the very recent report that applications of deoiled seed cake of Mahuva, Karanj, Neem and Castor as fertilizers to the saplings of *Lannea coromandelica* gave significant increases in growth of root, shoot, leaf area, number of branches and their total biomass production compared to the control plants (Naidu and Swamy, 1993).

4.2. Effect of neem cake on microbial populations in soil

4.2.1. Total bacteria

The data presented in Fig.2 relate to the total number of bacteria as influenced by single and two applications of neem cake. A significant decrease in bacterial population was observed in soil samples that received single application of neem cake when compared to unamended counterparts. The population of bacteria increased significantly with the age of the plant, raised
Fig. 2. Effect of single and two applications of neem cake on total number of bacteria in mulberry-grown soil.

The mean values ($n = 3$) of either single or two applications, indicated by bars, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

1. Soil samples, without or with a single application (0-day), were collected after 45 days (1) and 90 days (3) of plant growth.

2. Soil samples, without or with two applications (0 and 90 days), were collected after 45 days (2) and 90 days (4) of plant growth.
Figure 2

Bacterial population ($\times 10^7/g$ soil)

- 45 days
- 90 days

- Unamended
- Amended
either in unamended or neem cake-amended soil samples. But, no significant difference in population of bacteria, recorded after 45 and 90 days of plant growth was observed in soil samples collected from either unamended or amended (twice with neem cake) pots. On the contrary, Mishra et al. (1972) found that application of margosa or neem seed cake (MSC) to soil reduced the number of soil bacteria and actinomycetes.

4.2.2. Nitrifiers

Population of nitrifiers, in terms of their viable estimates, was also determined in mulberry-grown soil samples after 45 and 90 days of plant growth from both unamended and neem cake-amended (single or two applications) pots. Nitrifiers population decreased significantly in soil samples that received single application of neem cake compared to unamended samples (Fig.3). Thus, there was nearly a 50% reduction in population of nitrifiers from soil samples collected from neem cake-amended pots. And, this decrease in total population of nitrifiers proceeded further with the age of the plant. A similar trend was also observed with two applications of neem cake.

Likewise, Patil (1972) reported the inhibitory action of the non-edible oil cake of neem on nitrification. Mishra et al. (1975) observed that neem seed cake powder
Fig. 3. Effect of single and two applications of neem cake on nitrifiers population in mulberry-grown soil.

The mean values (n = 3) of either single or two applications, indicated by bars, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

- Soil samples, without or with a single application (0 day), were collected after 45 days (1) and 90 days (3) of plant growth.

- Soil samples, without or with two applications (0 and 90 days), were collected after 45 days (2) and 90 days (4) of plant growth.
Fig. 3

Nitrifiers population (MPN x 10^3/g soil)

- 45 days
- 90 days

Unamended Amended

Fig. 3
In soil incubation experiments inhibited the population of *Nitrosomonas*. Also, Khandelwal *et al.* (1977) observed that neem cake extracts at 1 and 2 ppm inhibited the *Nitrosomonas* for four weeks and *Nitrobacter* population for one week, respectively. Similarly, neem cake-amendment inhibited the action of *Nitrosomonas* and *Nitrobacter* in a black soil (Singh *et al*., 1980). Watanabe *et al.* (1981) reported reduction in losses of N-fertilizer by the inhibitory effect of neem cake-amendments to soil under flooded conditions. No attempt has been made in the present investigation to differentiate the two categories of nitrifiers, nitrosifying and nitrifying bacteria, in soil samples. It is clearly evident from the above results that the autotrophic nitrifiers are, in general, sensitive to the soil-applications of neem cake.

4.2.3. Population of *Azospirillum lipoferum*

An attempt was made initially to identify the species of *Azospirillum*, isolated from mulberry-grown soil samples from both unamended and neem cake-amended pots after 45 and 90 days of plant growth. The isolate of *Azospirillum* sp. obtained as white, dense and fine pellicle, developed a few millimeters below the surface of semi-solid malate medium within 36 hours at 37°C, was further purified by two or three transfers to the fresh malate
medium. All the isolates were characterized following the culture techniques described by Neyra and Doberelner (1977). The organisms possessed similar characteristics as described for *Azospirillum lipoferum* Beijerinck by Tarrand *et al.* (1978).

A significant increase in population of *Azospirillum lipoferum* was observed in neem cake-amended soil sample receiving single application compared to unamended sample at the end of 45 days of plant growth (Fig.4). However, no change in the population was evident after 90 days of plant growth. Two applications of neem cake also resulted in a similar observation in population density of *A. lipoferum*.

Similarly, neem cake-amendments were shown to stimulate the growth of blue-green algae in a flooded soil (Watanabe *et al.*, 1982). The present results indicate that the population of free-living diazotrophs such as *A. lipoferum* would greatly increase in the rhizosphere of mulberry under the influence of neem cake-amendments. However, a comparison of the data on nitrifiers (Fig.3) and *A. lipoferum* (Fig.4) clearly suggests that the population of microorganisms implicated in different transformations of nitrogen, respond differentially to the soil amendments of neem cake. Such a differential
Fig. 4. Effect of single and two applications of neem cake on *Azospirillium lipoferum* population in mulberry-grown soil.

The mean value \((n = 3)\) of either single or two applications, indicated by bars, followed by the same letter are not significantly different \((P < 0.05)\) from each other according to DMR test.

Soil samples, without or with a single application (0-day), were collected after 45 days (1) and 90 days (3) of plant growth.

Soil samples, without or with two applications (0 and 90 days), were collected after 45 days (2) and 90 days (4) of plant growth.
Azospirillum population (MPN x 10^5 soil)

Unamended Amended

Fig. 4
response, viz., inhibition of Nitrosomonas and Nitrobacter population, and stimulation of a nitrogen-fixing bacteria, Azotobacter was also noticed earlier (Ramanathan, 1983).

4.2.4. Population of fungi

Population of fungi increased significantly in soil samples that received single application of neem cake compared to unamended soil samples (Fig.5). Soil samples collected from posts receiving two applications of neem cake also showed similar trend in the population size of fungi. Applications of oil cakes of neem, groundnut, mahua and castor were shown to increase saprophytic fungi in the rhizosphere (Wajidkhan et al., 1979). Addition of neem cake and rock phosphate, alone or in combination, to the soil lowered the soil pH, and revealed that total nitrogen, available P₂O₅ and organic carbon increased with their addition (Singh, 1990). Thus, the increase in fungal population in neem cake-amended soil samples might be due to the low soil reaction.

4.3. Effect of neem cake on ammonification and nitrification in soil

4.3.1. Ammonification

The data on ammonification, as total nitrogen in the form of NH₄⁺, NO₂⁻ and NO₃⁻ under the impact of
Fig. 5. Effect of single and two applications of neem cake on fungal population in mulberry-grown soil.

The mean values (n = 3) of either single or two applications, indicated by bars, followed by the same letter are not significantly different (P ≤ 0.05) from each other according to DMR test.

- Soil samples, without or with a single application (0-day), were collected after 45 days (1) and 90 days (3) of plant growth.

- Soil samples, without or with two applications (0 and 90 days), were collected after 45 days (2) and 90 days (4) of plant growth.
Fig. 5

Fungal population (10^7/g soil)

45 days

90 days

Unamended Amended

Fig. 5
single and two applications of neem cake are presented in Table 4. In general, a significant decrease in mineralization of the added peptone-N was evident in soil samples that received either single or two applications of neem cake when compared to unamended soil samples. Mineralization of the added organic nitrogen in both unamended and neem cake-amended soil samples, collected after 45 and 90 days of plant growth, was rapid after 20 days of soil incubation. Thus, about 80-90% of added nitrogen was mineralized after 20 days of incubation. No change in the mineralization of peptone-N was observed in unamended soil samples either with age of the plant or soil incubation time with the organic nitrogen. However, a significant increase in the mineralization of added peptone-N was observed in amended soil samples, associated either with age of the plant or incubation time. This trend in mineralization of organic nitrogen continued even in soil samples that received two applications of neem cake.

The data presented in Figs. 6, 7 and 8 are related to the accumulation of $\text{NH}_4^+$, $\text{NO}_2^-$ and $\text{NO}_3^-$, respectively, at different time intervals under the influence of single or two applications of neem cake. The accumulation of ammonical nitrogen from the added organic nitrogen in the unamended soil samples significantly
Table 4. Effect of single (0-day) and two applications (0 and 90 days) of neem cake on ammonification in mulberry-grown soil.

<table>
<thead>
<tr>
<th>Neem cake treatment</th>
<th>45**</th>
<th>90**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10***</td>
<td>20***</td>
</tr>
<tr>
<td></td>
<td>Unamended</td>
<td>Amended</td>
</tr>
<tr>
<td>Single application</td>
<td>863c</td>
<td>776a</td>
</tr>
<tr>
<td>Two applications</td>
<td>866bc</td>
<td>733a</td>
</tr>
</tbody>
</table>

* Values are μg (NH$_4^+$ + NO$_2^-$ + NO$_3^-$) - N g$^{-1}$ soil.

** Time, in days, of soil collection after neem cake application.

*** Time, in days, of soil sampling after incubation with 1000 ppm N peptone.

The mean values (n = 3), in a row, followed by the same letter are not significantly different (P ≤ 0.05) from each other according to DMR test.
Fig. 6. Effect of (A) single (0-day), and (B) two applications (0 and 90 days) of neem cake on ammonical nitrogen \( (NH_4^+ - N) \) formation in mulberry-grown soil.

The mean values \((n = 3)\), indicated by bars, followed by the same letter are not significantly different \((P \leq 0.05)\) from each other according to DMR test.

. Soil samples, without or with neem cake-amendment, were collected after 45 days of plant growth and supplemented with peptone \((1000 \text{ ppm } N)\) and incubated for 10 days (1) and 20 days (2).

. Soil samples, without or with neem cake-amendment, were collected after 90 days of plant growth and supplemented with peptone \((1000 \text{ ppm } N)\) and incubated for 10 days (3) and 20 days (4).
Fig. 6

(A) 

NH$_4^+$-N (µg/g soil) 

(B) 

NH$_4^+$-N (µg/g soil)
Fig. 7. Effect of (A) single (0-day) and (B) two applications (0 and 90 days) of neem cake on nitrite nitrogen ($\text{NO}_2^-$-N) formation in mulberry-grown soil.

The mean values ($n = 3$), indicated by bars, followed by the same letter are not significantly different ($P < 0.05$) from each other according to DMR test.

. Soil samples, without or with neem cake-amendment, were collected after 45 days of plant growth and supplemented with peptone (1000 ppm N) and incubated for 10 days (1) and 20 days (2).

. Soil samples, without or with neem cake-amendment, were collected after 90 days of plant growth and supplemented with peptone (1000 ppm N) and incubated for 10 days (3), and 20 days (4).
Fig. 7
Fig. 8. Effect of (A) single (0-day) and (B) two applications (0 and 90 days) of neem cake on nitrate nitrogen \( \text{NO}_3^- - \text{N} \) formation in mulberry-grown soil.

The mean values \( (n = 3) \), indicated by bars, followed by the same letter are not significantly different \( (P \leq 0.05) \) from each other according to DMR test.

Soil samples, without or with neem cake-amendment, were collected after 45 days of plant growth and supplemented with peptone (1000 ppm N) and incubated for 10 days (1) and 20 days (2).

Soil samples, without or with neem cake-amendment, were collected after 90 days of plant growth and supplemented with peptone (1000 ppm N) and incubated for 10 days (3) and 20 days (4).
decreased with increasing periods of soil incubation (Fig. 6). A significant decrease in accumulation of $\text{NH}_4^+$-N was observed in neem cake-amended soil samples when compared to unamended soil samples. This inhibitory action of neem cake towards the activity of ammonifying microorganisms in soil may possibly be due to inhibition of urease activity (Khandelwal et al., 1977). But accumulation of $\text{NH}_4^+$-N, from the added organic nitrogen in both unamended and neem cake-amended soil samples, significantly increased with the age of the plant. Two applications of neem cake resulted in a significant increase in mineralization of peptone-N when compared to single application. The accumulation of nitrite nitrogen from added organic nitrogen was more with increased periods of incubation both in unamended and neem cake-amended soil samples (Fig. 7). Compared to unamended soil samples, there was a significant decrease in the accumulation of $\text{NO}_2^-$-N in amended soil samples. Though a similar trend was observed with the two applications of neem cake, a significant decrease in the accumulation of $\text{NO}_2^-$-N after 10 days and an increase after 20 days were evident in soil samples. Nitrate nitrogen increased significantly with increased period of incubation both in unamended and neem cake-amended soil samples (Fig. 8). In general, the trend in the accumulation of $\text{NO}_3^-$-N was similar to that of nitrite nitrogen in soil samples amended with neem cake.
Soil treated with ammonium sulphate and 12% neem oil was found to exert a maximum inhibition in the formation of ammonium nitrogen and this inhibitory effect was attributed to the presence of some secondary chemical constituents or lipid associates (Patil, 1972). Margosa (neem) seed cake checked the conversion of ammonium to nitrite presumably by its selective effects on the ammonium-oxidizing bacteria (Mishra et al., 1975). According to Singh et al. (1979), blending of neem cake with nitrogen fertilizer, urea, seems to have slowed down the release of nitrogen, and prevented leaching losses of ammonia, owing to the presence of organic materials in the form of neem seed-cake. The reduction in ammonium volatilization due to linseed oil and neem oil was attributed to the slow release of ammonium ions (Singh, 1983). The use of organic compounds like p-benzoquinone, catechol, linseed and Azadiracta indica oils reduced leaching losses of NH$_4^+$-N and NO$_3^-$-N (Singh, 1985). A recent report (Thilmakapura, 1993) indicates that neem cake retarded the activity of nitrifying bacteria, which convert complex nitrogen compounds present in urea and ammonium fertilizers into useless nitrogen gas that gets liberated into the atmosphere as well as into highly soluble nitrates that are leached out of the soil.

4.3.2. Nitrification

The results in Table 5 show the impact of
Table 5. Effect of single (0-day) and two applications (0 and 90 days) of neem cake on nitrification* in mulberry-grown soil.

<table>
<thead>
<tr>
<th>Neem cake treatment</th>
<th>45**</th>
<th>90**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10***</td>
<td>20***</td>
</tr>
<tr>
<td></td>
<td>Unamended</td>
<td>Amended</td>
</tr>
<tr>
<td>Single application</td>
<td>126b</td>
<td>110a</td>
</tr>
<tr>
<td>Two applications</td>
<td>112d</td>
<td>84b</td>
</tr>
</tbody>
</table>

* Values are μg (NO$_2^-$ + NO$_3^-$) - N g$^{-1}$ soil.

** Time, in days, of soil collection after neem cake application.

*** Time, in days, of soil sampling after incubation with 200 ppm N ammonium sulphate.

The mean values (n = 3), in a row, followed by the same letter are not significantly different (P < 0.05) from each other according to DMR test.
single or two applications of neem cake on nitrification, presented as total nitrogen in the form of \( \text{NO}_2^- \)-N and \( \text{NO}_3^- \)-N. The oxidation of added inorganic nitrogen, as ammonium sulphate, was inhibited in neem cake-amended soil samples compared to unamended samples. The accumulation of both \( \text{NO}_2^- \)-N and \( \text{NO}_3^- \)-N decreased with increasing periods of incubation. A similar trend was also observed with two applications, but the oxidation of inorganic nitrogen decreased in unamended soil samples with increasing age of the plant. The extent of \( \text{NO}_2^- \)-N and \( \text{NO}_3^- \)-N accumulation as result of the activities of nitrosifying and nitrifying bacteria, is shown in Figs. 9 and 10, respectively. The formation of either \( \text{NO}_2^- \)-N or \( \text{NO}_3^- \)-N in soil samples is directly related to the accumulation of total nitrogen.

The available information indicates that the neem cake is inhibitory to the nitrification in soils. For instance, increase in the level of neem oil from 1.5 to 12% correspondingly decreased the nitrification rate (Patil, 1972). Evidently, the oil content of the neem cake inhibits the activity of nitrifiers in soil. However, margosa (neem) seed-cake delayed nitrification for 6 weeks with its addition, at the rate of 0.2% C, and nitrate formation was delayed for 28 and 90 days when neem cake was added at the rate of 0.2% and 2% C, respectively (Mishra et al., 1975). The alcohol extracts of neem cake
Fig. 9. Effect of (A) single (0-day) and (B) two applications (0 and 90 days) of neem cake on nitrite nitrogen ($\text{NO}_2^{-}\text{-N}$) formation in mulberry-grown soil.

The mean values ($n = 3$), indicated by bars, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

Soil samples, without or with neem cake-amendment, were collected after 45 days of plant growth and supplemented with ammonium sulphate (200 ppm N) and incubated for 10 days (1) and 20 days (2).

Soil samples, without or with neem cake-amendment, were collected after 90 days of plant growth and supplemented with ammonium sulphate (200 ppm N) and incubated for 10 days (3) and 20 days (4).
Fig. 9
Fig. 10. Effect of (A) single (0-day) and (B) two applications (0 and 90 days) of neem cake on nitrate nitrogen (NO$_3^-$-N) formation in mulberry-grown soil.

The mean values ($n = 3$), indicated by bars, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

Soil samples, without or with neem cake-amendment, were collected after 45 days of plant growth and supplemented with ammonium sulphate (200 ppm N) and incubated for 10 days (1) and 20 days (2).

Soil samples, without or with neem cake-amendment, were collected after 90 days of plant growth and supplemented with ammonium sulphate (200 ppm N) and incubated for 10 days (3) and 20 days (4).
Fig. 10
at the rate of 30% N was effective in retardation of nitrification up to 45 days (Sahrawat, 1975). According to a recent study, the concentration of $\text{NO}_3^-$-N in soils of the plots which received N through neem cake blended urea was lower at the initial stages of growth of wheat, and was higher at the stages of growth as compared with the concentration of $\text{NO}_3^-$-N in soils of the plots fertilized with urea alone (Singh et al., 1986). Triterpenes, the useful compounds in neem cake checks the nitrification process, and provides the plants with more nitrogen from the same amount of fertilizers (Thimakapura, 1993).

4.4. Effect of neem cake on enzyme activities in soil

4.4.1. Amylase

The activity of soil enzyme, amylase, was determined in unamended and neem cake-amended soil samples, at 45 and 90 days of plant growth. Amylase activity was measured by amending the soil with starch and incubating for 24 or 72 hrs at 28°C. The accumulation of glucose was significantly more both in unamended and neem cake-amended soil samples, and progressively increased with increasing age of mulberry (Fig.11). Glucose accumulation significantly increased in neem cake-amended soil samples compared to unamended soils. The enzyme activity seems to have increased with the age of
Fig. 11. Effect of (A) single (0-day) and (B) two applications (0 and 90 days) of neem cake on amylase activity in mulberry-grown soil.

The mean values (n = 3), indicated by bars, followed by the same letter are not significantly different (P ≤ 0.05) from each other according to DMR test.

Soil samples, without or with neem cake-amendment, were collected after 45 days of plant growth and supplemented with starch (2% W/W) and incubated for 24 hrs (1) and 72 hrs (2).

Soil samples, without or with neem cake-amendment, were collected after 90 days of plant growth and supplemented with starch (2% W/W) and incubated for 24 hrs (3) and 72 hrs (4).
Fig. 11

(A) Glucose accumulated (μg/g soil) over 45 and 90 days.

(B) Glucose accumulated (μg/g soil) over 45 and 90 days.

Legend:
- Unamended
- Amended
the plant as well as with the incubation time. Excepting for the significant increases in the amount of glucose, associated with each treatment and sampling time, the activity of amylase as a response to single and two applications of neem cake remained same.

4.4.2. Invertase

As with the amylase activity, the influence of neem cake-amendments (single or two applications) towards the activity of invertase, measured as glucose formed from sucrose, proceeded in the same pattern (Fig.12). Of interest was the decrease in the activity of invertase in unamended soil samples even with increased age of the plant or incubation time of sucrose-amended soil samples. Again, although the response in enzymatic activity of soil samples that received two applications of neem cake was similar to that of single application, comparatively two applications decreased the extent of enzyme activity.

4.4.3. Protease

Protease activity in soil samples, measured as amount of tyrosine formed from casein after 24 hrs incubation at 30°C, also responded to the applications of neem cake in a way similar to that of amylase activity. Thus, an increase in the activity of enzyme was observed in neem cake-amended soil samples compared to unamended soil (Fig.13).
Fig. 12. Effect of (A) single (0-day) and (B) two applications (0 and 90 days) of neem cake on invertase activity in mulberry-grown soil.

The mean values (n = 3), indicated by bars, followed by the same letter are not significantly different (P < 0.05) from each other according to DMR test.

. Soil samples, without or with neem cake-amendment, were collected after 45 days of plant growth and supplemented with 18 mM sucrose and incubated for 24 hrs (1) and 48 hrs (2).

. Soil samples, without or with neem cake-amendment, were collected after 90 days of plant growth and supplemented with 18 mM sucrose and incubated for 24 hrs (3) and 48 hrs (4).
Glucose accumulated (µg/g soil)

45 days

90 days

Unamended HHl Amended

Fig. 12
**Fig. 13.** Effect of single and two applications of neem cake on protease activity in mulberry-grown soil.

The mean values \((n = 3)\) of either single or two applications, indicated by bars, followed by the same letter are not significantly different \((P<0.05)\) from each other according to DMR test.

- Soil samples, without or with a single application (0-day) of neem cake, were collected after 45 days (1) and 90 days (3) of plant growth and supplemented with casein (2% W/V) and incubated for 24 hrs.

- Soil samples, without or with two applications (0 and 90 days) of neem cake, were collected after 45 days (2) and 90 days (4) of plant growth and supplemented with casein (2% W/V) and incubated for 24 hrs.
Fig. 13

Tyrosine formed (μg/g soil)

Unamended Amended

Fig. 13
4.4.4. Phosphatase

The data presented in Fig. 14 show the impact of neem cake-amendments in mulberry cultivation on the activity of soil phosphatase, determined as the amount of $p$-nitrophenol released from $p$-nitrophenol phosphate after 3 hrs incubation. Interestingly, the activity of phosphatase significantly decreased in neem cake-amended soil samples compared to unamended soils. The reason for such a decline in the activity of phosphatase in soils treated with neem cake is not clearly understood. This trend of depressed activity of phosphatase also was evident with two applications of neem cake.

4.4.5. Urease

The response of urease towards applications of neem cake to soil samples (Fig. 15) is similar to that of phosphatase activity (Fig. 14). The amount of ammonia formed from the substrate, urea, after 3 h soil incubation at 37°C was significantly less in neem cake-amended soil samples compared to unamended soil samples. This is true even with two applications of neem cake. The data on the influence of soil amendment of neem cake towards ammonification (Table 4) and urease activity (Fig. 15) clearly suggest that the accumulation of $NH_4^+$ by the activity of ammonifying microorganisms is in direct relation to the production of urease in soil. Evidently,
Fig. 14. Effect of single and two applications of neem cake on phosphatase activity in mulberry-grown soil.

The mean values \( (n = 3) \) of either single or two applications, indicated by bars, followed by the same letter are not significantly different \( (P \leq 0.05) \) from each other according to DMR test.

- Soil samples, without or with a single application (0-day) of neem cake, were collected after 45 days (1) and 90 days (3) of plant growth and supplemented with p-nitrophenol phosphate \( (0.03 \text{ M}) \) and incubated for 3 hrs.

- Soil samples, without or with two applications (0 and 90 days) of neem cake, were collected after 45 days (2) and 90 days (4) of plant growth and supplemented with p-nitrophenol phosphate \( (0.03 \text{ M}) \) and incubated for 3 hrs.
Fig. 14

PNP accumulated (µg/g soil)

Unamended Amended

45 days 90 days
Fig.15. Effect of single and two applications of neem cake on urease activity in mulberry-grown soil.

The mean values \((n = 3)\) of either single or two applications, indicated by bars, followed by the same letter are not significantly different \(\left( P \leq 0.5 \right)\) from each other according to DMR test.

- Soil samples, without or with a single application \((0\text{-day})\) of neem cake, were collected after 45 days \((1)\) and 90 days \((3)\) of plant growth and supplemented with urea \((3\% \text{ W/W})\) and incubated for 3 hrs.

- Soil samples, without or with two applications \((0 \text{ and } 90 \text{ days})\) of neem cake, were collected after 45 days \((2)\) and 90 days \((4)\) of plant growth and supplemented with urea \((3\% \text{ W/W})\) and incubated for 3 hrs.
Fig. 15

Ammonia accumulated (µg/g soil)

<table>
<thead>
<tr>
<th></th>
<th>45 days</th>
<th>90 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unamended</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amended</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
the decreased activity of ammonifying organisms in neem cake-amended soil samples was due to the depressed activity of urease as suggested Khandelwal et al. (1977).