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Review

Effects of nitrogen fertilization on crops and their associated insect communities: a global food security perspective

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Abstract: Nitrogen (N) fertilization reduces worldwide food insecurity by boosting crop yield and stability. N is one of the most essential macromolecules required for the growth and reproduction of plants. It occurs in diverse chemical forms and circulates in natural and agricultural ecosystems. It is a constituent of chlorophyll, hence is required for the photosynthesis of plants. Plants receive N through their roots in the form of ammonia or nitrate. Nutritional quality and defense of plants that have a direct impact on herbivorous insects are altered by N fertilization and herbivorous insects can differentiate between plants that receive different applications of N fertilizer. Increasing N fertilization has a variable impact on plant species composition, plant growth, plant biomass, and yields. Plant tissue N and protein contents are also affected by nitrogen fertilization. Moreover, nitrogen fertilization affects many aspects of insects such as population dynamics, larval count, larval weight, feeding choice, and oviposition preference. Furthermore, predatory insect abundance, parasitization performance, and development of parasitoids on host insects are negatively affected by N fertilization. Other important effects of N fertilization are the hemolymph protein profile of herbivores, emission of VOCs, phytohormone biosynthesis, and direct and indirect defense of plants. The aim of this literature research is to demonstrate the effects of variable doses of N fertilization on the crop-herbivore-natural enemy tri-trophic systems. The information gathered in this review might help researchers understand the impact of optimal and excessive N fertilization on crop production and food security.

Keywords: nitrogen fertilization; crops; insect communities; food security

1. Introduction

A significant worldwide worry now centers on how modern agriculture affects natural resources. The increasing pressure on land and water resources is a result of population growth and rising consumer demand for agricultural products. The inefficient use of resources, particularly nitrogen (N), in many intensively managed agricultural systems with large external inputs is a key cause for worry. Almost 50% of the people on the earth currently depend on food produced as a result of synthetic nitrogen fertilizers (Erisman *et al.*, 2008; Smil 2002 and Stewart *et al.*, 2005, Ritchie *et al*., 2022) (Figure 1).

It is well-recognized that one of the most important macromolecules for plant development and reproduction is N. It occurs in various chemical forms and flows around natural and agro-ecosystems in a cyclical way. It is a basic element of chlorophyll and hence it is obligatory for the photosynthesis of plants (Prsa *et al.*, 2007). N is taken up by plants by means of their roots in the form of nitrate or ammonia. Generally, mineral fertilizers are used to enhance the yield and biochemical characteristics of agricultural crops (Akther *et al*., 2020; Basak *et al*., 2016; Bentz *et al*., 1995; Hossain *et al*., 2015; Islam *et al*., 2019; Jahan *et al*., 2020; Sarker *et al*., 2017; Shariot-Ullah *et al*., 2016.). However, the N cycle of the ecosystem has been considerably altered by human beings through the application of agricultural fertilizers. Application of excess N fertilizer not only increases the cost of agricultural production but also can pose threat to the environment and influence the pest responses to the crops and their natural enemies. The nutritional quality and defense of plants are altered by N fertilization (Chen *et al.*, 2008b). So, considering the ecological and economic reasons, fertilizers should be applied carefully for the optimal growth of plants.

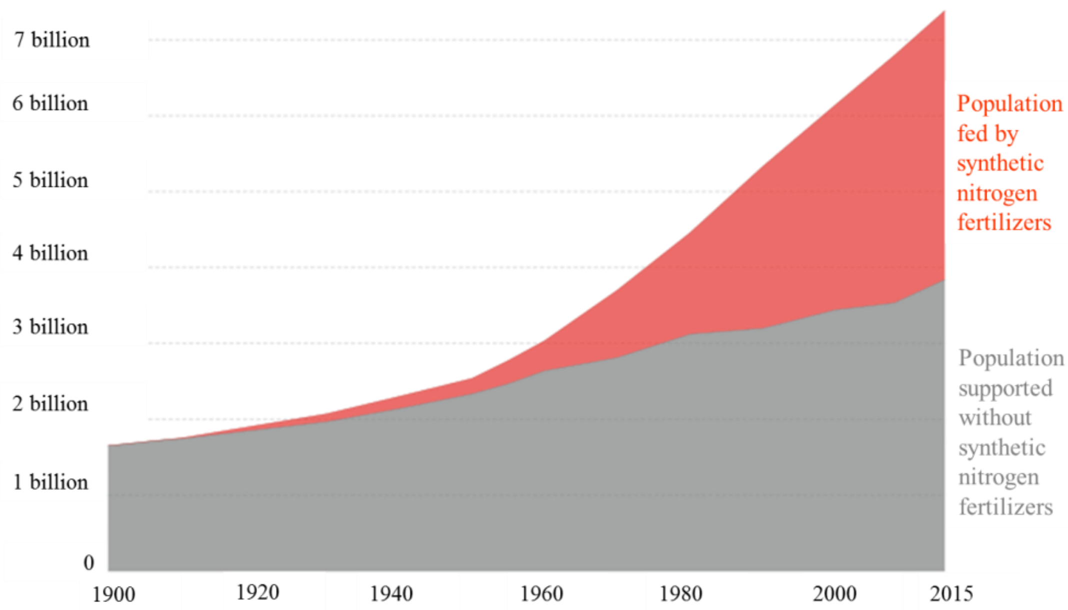

Figure 1. World population with and without nitrogen fertilizers (adapted from Erisman *et al.*, 2008; Smil, 2002; Stewart *et al*., 2005; Ritchie *et al*., 2022).

Although N fertilization increases the total growth of vegetation, it reduces plant species richness and abundance of host plants. The abundance of herbivores is decreased by N fertilization and the parasitoids depend on herbivore abundance. The underlying reasons for reduced herbivore abundance caused by N fertilization are lower host plant abundance and stalk number and altered patterns of localization of hosts within higher vegetation. Moreover, fertilization also negatively affects the third trophic level by cascading up through the abundance of hosts. In this way, the tri-trophic system i.e., the plant-herbivore-natural enemy system is affected which impedes the conservation and management of multi-trophic systems (Chen *et al.*, 2014).

Alteration of natural habitats to agricultural fields has enhanced drastically during the last century. This intensification of agrological practices particularly in the form of fertilization is one of the main factors that might explain why a number of flora and fauna are endangered. Several literatures (Chen and Ruberson, 2008; Chen *et al.*, 2014; Veromann *et al.*, 2013) suggest that these alterations are detrimental to biodiversity. It is, therefore, necessary to investigate the interactions among plants, herbivorous insects and insect natural enemies which might take place in the altered agro-ecosystem to judge the impact of anthropogenic conversion of natural habitat.

In addition to plant species richness, an individual plant might be affected by N-fertilization. For example, N content and plant secondary metabolites that play important role in herbivory are also altered by N fertilization (Veromann *et al.*, 2013). However, the effect of N fertilization on plant and insect communities in agro-ecosystems has been poorly understood. Hence, the objectives of this review are to find out the effect of N fertilization on different aspects of plants, insect pests, and their natural enemies.

2. Effect of nitrogen fertilization on crops

2.1. Effect on growth parameters and yield

The application of elevated N affects different growth parameters of plants. For instance, Jang *et al.* (2008) studied the effect of elevated N levels on growth parameters of three rice (*Oryza sativa*) cultivars i.e., *cv. Daesanbyeo*, *cv. Dongjinbyeo* and *cv. Junambyeo*. The N fertilizer was applied in the form of urea at three different rates i.e., 0.00, 36.8, and 73.6 kg/ha N. Enhanced application of N up to the first-rate only (36.8 kg/ha) increased plant height and shoot length in all three rice cultivars. It indicates that a high application rate of urea can increase the growth of rice up to a threshold level after which the growth declines. Similarly, applications of N at the rate of 36.8 kg/h enhanced both plant dry weight and shoot dry weight as compared to the control, but significantly decreased with double N dose (73.6 kg/ha). The above results might be due to a progressive decrease of enzymatic (nitrate reductase) activity at the very high concentration of N (Jang *et al.,* 2008). Jahan *et al.* (2019) assessed the effects of nitrogen on the growth and yield of two rice cultivars, IRRI dhan58 and IRRI dhan75, using dosages of 0, 25, 50, 75, 100, 125, 150, and 175 kg/ha and revealed similar results. According to their findings, application of N at the rate of 125 kg/ha and 75 kg/ha produced the highest grain yields for the IRRI dhan58 (7.30 ton/ ha) and IRRI dhan75 (4.82 ton/ ha), respectively. Hossen *et al*. (2016) investigated the effects of nitrogen on the seed yield of the mungbean, *Vignaradiata* (L.), using five different nitrogen dosages, namely 0, 30, 45, 60, and 75 kg/ha. They discovered that the application of nitrogen doses significantly increased the seed yield, with the highest yield being 1.85 ton/ha in 45 kg/ha N. Thus the crop yield yield initially increased with N application up to a specific point before declining with higher N rates.

Similar results were also obtained by applying four different doses of N (0, 60, 120, and 180 kg/ ha) against three cotton cultivars (NIAB-111, CIM- 496, and FH- 901) (*Gossypium* sp.) (Saleem *et al.,* 2010). The highest seed cotton production (3083.4 kg/ha) was reported for N at a rate of 60 kg/ha, which differed considerably from all other levels, while the lowest yield (2765.4 kg) was observed in control. Another study (Shah *et al.,* 2021) reported that low N application rates combined with high plant densities (120 kg/ha N and 12 plants/m^2) or high N application rates combined with medium plant densities (180 kg/ha N and 10 plants/m^2) produce higher seed cotton. Production of siliques in crucifers is also affected by N fertilization. Veromann *et al.* (2013) reported that the number of siliques and yield of *Brassica napus* were significantly increased by N fertilization. The least amount of siliques and the lowest yield were obtained from control plants that did not receive external N. Treatments of 100 to 160 kg/ha N produced the most siliques, although there were no appreciable differences between them. It is, therefore, evident that crop yield first rises with N application up to a certain point before falling with higher N rates.

2.2. Effect on nitrogen and chlorophyll content

Nitrogen content in plant tissues varies depending on the application of different doses of nitrogen fertilizers. This plant tissue N affects the abundance of insects feeding on plant tissues. A previous study showed that there is a significant and positive correlation between herbivore abundance and plant tissue N (Haddad *et al.,* 2000). In another study (Chen *et al.* ,2008a) cotton plants were treated with different concentrations of N viz. 19, 50, 87, and 125 kg/ha to observe their effect of cotton tissue N. The amount of tissue N on leaf blades varied based on different doses of N. Although no significant differences were found regarding the amount of total N treated with 87 and 125 kg/ha N, plants treated with 87 N/ha had higher percentages of total N compared to those treated with 50 kg/ha N. It was also true for 50 kg/ha when compared with 19 kg/ha N. Thus, increased N fertilization enhanced the leaf blade's total N content. Similarly, leaf petiole nitrate-N, an indicator of cotton N status increased with increasing N fertilization. The average value of nitrate-N found in ppm was 14416.60, 8120.40, 1622.00, and 163.40 against 19, 50, 87, and 125 kg/ha N treatment, respectively. Increased nitrogen level in response to elevated nitrogen treatment on ‘Golden Delicious’ apple trees was also reported by Prsa *et al.* (2007).

Chlorophyll, the green pigment in plants, is one of the most active pigments responsible for photosynthesis. Apart from bacteria, which have chlorophyll *a* and chlorophyll *b*, there are currently at least nine known chlorophylls, and these are the chlorophylls that are the most prevalent and well-known in all autotrophic species (Kacar *et al.,* 2002). The primary cause of plants becoming yellow in the absence of nitrogen is the breakdown of proteins, followed by the breakdown of plastids, which causes chlorophyll synthesis to either slow down or stop (Kacar *et al.,* 2002). It is suggested that there is a close connection between nitrogen fertilization and the amount of chlorophyll in the leaves (Odabas, 1981).

In a recent study, barley (*Hordeum vulgare* L.) was fertilized with N at rates of 0, 40, and 80 kg/ha. The rate that produced the maximum chlorophyll content was 80 kg/ha N (Hamann *et al*., 2020). In a previous study N dosages of 0, 80, and 250 kg/ha were used to determine the effects of N administered in various ratios on the nitrogen and chlorophyll concentrations in the leaves of Golden Delicious apples, and chlorophyll values were specified throughout five distinct periods (Prsa *et al.,* 2007). Chlorophyll content was significantly higher in the leaves treated with 250 kg/ha compared to the control treatment. But during the growing season of the plants, the same amount of chlorophyll was found in leaves treated with 80 kg/ha indicating that the leaf chlorophyll level not only depends on N fertilization, but also on leaf thickness, which increased during the growing season (Prsa *et al.,* 2007). Similar findings were also reported in another investigation, demonstrating that higher nitrogen doses were associated with a rise in chlorophyll levels (Uysal, 2018). In another study, immature Rocha pears that had not yet produced any fruits were given N dosages of 0, 10, 20, and 40 kg/ha. The amount of chlorophyll in leaf samples collected 160 days after full blooming was determined. According to the findings of the study, the application with no nitrogen had the lowest chlorophyll content, while the other three applications examined in the same group had higher chlorophyll content (Neto *et al.,* 2011).

3. Effects of nitrogen fertilization on insects

3.1. Effects on herbivorous insects

Herbivorous insects feed on plant tissues or plant products. Nitrogen fertilization affects many aspects of herbivorous insects like population dynamics (Chen and Ruberson, 2008; Schowalter, 2016), larval count (Veromann *et al.,* 2013), larval weight, larval development, feeding choice, and oviposition preference (Chen *et al.,* 2008a; Veromann *et al.,* 2013). It is well established that N at optimal doses is good to plant growth, but excessive use of N fertilizer is harmful for plants (Elhanafi *et al*., 2019). In Asia, the brown plant hopper (BPH), *Nilaparvata lugens* (Stål) is one of the most significant insect pests of rice. The fitness features of BPH are known to be impacted by variations in nitrogen fertilizer inputs to rice plants (Rashid et al., 2017). The survival rate of BPH from egg to nymph or adult stages rises with increasing nitrogen input. Furthermore, too much nitrogen boosts BPH growth and adds to new body weight. Plants that receive more nitrogen fertilizers collect more nitrogen and soluble protein in their tissue, which ultimately affect the growth and development of herbivores (Rashid *et al*., 2017). Besides, with higher nitrogen levels, egg hatchability increases, which leads to more BPH being produced with heavier dry body weights. Thus, all fitness features of BPH are increased by increasing nitrogen input (Rashid *et al.,* 2017). Additionally, research has shown that BPH favours nitrogen-rich plants over those with low nitrogen levels (Lu *et al.,* 2005). On the other hand, rice plants with low nitrogen levels make more constitutive Silicon, a vital defense molecule in rice plants, and more insect-defensible soluble phenolics (De, 1936; Inbar *et al.,* 2001; Larbat *et al.,* 2012; Stout *et al.,* 1998). Another harmful migratory pest of rice, *Sogatella furcifera* (Horváth), is said to be affected by nitrogen fertilization. For example, higher nitrogen fertilizer application to rice plants increases the feeding and oviposition preferences of adults, and nitrogen fertilizer application increases the longevity of the adults and reduces the generation reproduction time of *S. furcifera*. As a result, changing nitrogen inputs to rice plants have a bottom-up impact on the biological characteristics of *S. furcifera* which may help to explain why *S. furcifera* infests a high percentage of rice paddies that get high nitrogen fertilizer levels (Li *et al.,* 2021). Population dynamics of insect is changed in response to N treatment (Chen and Roberson, 2008). Increased N fertilization has been reported to cause a significant increase in the mirid population. Throughout the season, the mirid population remains significantly lower with lowest N treatment, and during the late season the mirid population increases with higher N treatment, but a significant decrease of population of aphids, *Aphis gossypii* occurs. This incompatible result is explained by several factors such as environmental variables, natural enemies, and defensive responses of plants to aphid feeding. For instance, most predatory insects like aphidophagous coccinellids might play a significant role in mediating aphid response to N. In a recent study, Veromann *et al.* (2013) investigated the effect of different doses of N fertilizers on the oviposition preference of *Meligethes aeneus* on flowers of *Brassica napus* and found that plants treated with low and high N levels were more attractive to herbivorous insects than plants receiving moderate N levels. Since there was a monotonic increase of flowers and consequently the accessibility of proper oviposition and feeding places with the addition of N, it could be assumed that host plant selection by herbivores was more affected by info chemicals compared to flower abundance. The amount of nitrogen content also varies with the application of nitrogen fertilizers. For instance, a previous study (Bentz *et al.,* 1995) found significantly more N in the tissues of parasitized whitefly pupae than the non-parasitized. Moreover, significantly higher N was recorded in pupae reared on NH_4NO_3 treated plants compared to $Ca(NO_3)_2$ treated plants. This higher N content of whitefly pupae on NH_4NO_3 treated plants might have made the pupae less suitable than those on $Ca(NO_3)_2$ treated plants. Hence, enhanced oviposition by *E. formosa* on *B. argentifoli* was observed on $Ca(NO_3)_2$ treated plants. Furthermore, protein-N content was higher in plants treated with NH_4NO_3 (11.53%) than with plants treated with $Ca(NO_3)_2$ (10.58%) and control plants (6.70%).

3.2. Effects on insect natural enemies

Natural enemies of insect pests include predators, parasitic insects, and parasitoids. Insect natural enemies are affected by N fertilization. The literature revealed that predatory insect abundance (Chen *et al.,* 2014), parasitization performance (Veromann *et al.,* 2013), and development of parasitoids on host insects (Chen *et al.,* 2014) are negatively affected by N fertilization. Chen *et al.* (2008) described the effect of different doses of N on beneficial insect populations for 2 consecutive years. Results showed that the abundance of predatory insect populations, particularly the population of spiders was significantly affected by N treatment in the first year. A higher spider population was counted with the highest N treatment. There was no significant differences between the number of spiders in the three lower N applications (no fertilizer, 45 kg/ha and 90 kg/ha, respectively) and also two higher N applications (90 kg/ha and 135 kg/ha, respectively). Significant interactions were found between the sampling date and N on lady beetles. In the following year, only the predatory bug, *Geocoris* spp. was significantly affected by N treatment. This species seemed to dominate throughout the growing season but the highest abundance of this species was in the mid-season and lowest in the lowest N treatments (0 kg/ha N). Although there were no significant differences in the populations between treatments in 45, 90, and 135 kg/ha N, all of those were significantly higher than those in the 0 kg/ha N. Predatory coccinellids and chrysopids were significantly affected by sampling dates.

The parasitization rate is also affected by differential doses of N treatment. Chen *et al*. (2008) found the highest parasitism rate of caterpillars in the cotton, (*G. hirsutum*, the major source of cottonseed oil) plots treated with 0 kg/ha N in the first year, whereas in the second year highest parasitism rate was found in the plots treated with 135 kg/ha N. However, the recovery rates of caterpillars observed were low in both years. It was assumed that most of the caterpillars were predated or lost, which might have an effect on the rate of parasitism if the unparasitized and parasitized caterpillars were predated at different rates. Bentz *et al.* (1995) reported the effect of N fertilizers as NH_4NO_3 (33.5% total N), $Ca(NO_3)_2$ (15.5% total N), and a control group with no N on the parasitization of *Bemisia argentifoli* by the wasp *Encarsia formosa* reared on the plant poinsettia *Euphorbia pulcherrima*. The treatment consisted of two tests: in a choice test each plant infested with whitefly were treated with NH_4NO_3, $Ca(NO_3)_2$ or control and exposed to wasps; in a non-choice test each plant infested with whitefly were exposed to wasps. A higher number of wasps was found on fertilized plants compared to control plants resulting in the following rank: NH_4NO_3 > $Ca(NO_3)_2$ > control plants. The underlying reason of the higher number of wasps on NH_4NO_3 treated plants over $Ca(NO_3)_2$ treated plants was assumed to be due to preferential probing by *E. formosa* on high N, green and vigorous plants.

The effect of N fertilization on the development of parasitoids in host insects has been described by Chen *et al.* (2014). They reported that although the percentage of the host insect, *Spodoptera exigua* larvae yielding the parasitoid, *C. marginiventris* cocoons are known to be unaffected by N treatments, the percentage of *C. marginiventris* cocoons developed to adults in *Spodoptera exigua* larvae at 112 and 280 ppm N treatment levels was two times higher than both 42 and 196 ppm N-treatments. However, the mechanisms underlying this effect on *C. marginiventris* is not clear. Moreover, the developmental period of *C. marginiventris* offspring egg to pupal development and to the emergence of the adult is significantly reduced with increasing N treatment. Although no significant influence was observed on right meta-thoracic tibia length (RMTL) and size of the male parasitoids, positive and a significant correlation was found between N application rate and RMTL.

4. Other related effects of N fertilization on crops and insects

4.1. Effects on emission of volatile organic compounds

Nearly all plant taxa naturally produce volatile organic compounds (VOCs). VOCs emission occurs in plants in response to insect herbivory (Chen *et al.*, 2008b) which play an important role in finding host-plants by other herbivorous insects (Veromann *et al.*, 2013) and host-herbivorous insects by parasitoids (Olson *et al.*, 2009; Veromann *et al.*, 2013). The emission of VOCs is profoundly affected by N-fertilization. Veromann *et al.* (2013) identified 19 different VOCs in N-treated *Brassica napus* among which emission of acetic acid, α-pinene, 3-carene, and benzaldehyde were at higher levels from both flower and bud stages. However, the release of some compounds particularly α-thujene, LOX, and acetic acid increased considerably with increased N availability. Significant correlations were found between bud-stage VOCs and *M. Aeneus* larval abundance. The abundance of *M. Aeneus* Larvae was positively correlated with (3Z)-hexenyl acetate, 3-carene, camphene, limonene, acetic acid, and indole emission rates, but negatively correlated with ß-pinene, (E,E)-α- farnesene, benzaldehyde and methyl benzoate. Therefore, on the basis of the direction of the correlation, these VOCs could be recommended as attractants or repellents for female *M. Aeneus.* Some bud-stage VOCs particularly α-pinene, ß-pinene, and camphene had significant but weak correlations with the abundance of *C. obstrictus* damaged siliques and the abundance of *C. obstrictus* was very low. The highest infection rate of siliques was 14.3%, much lower than the threshold rate (26%) for significant yield losses. Chen *et al*. (2008b) also described the impact of different rates of N-fertilization (42, 112, 196, and 280 ppm) on emission rates of VOCs from herbivore-damaged cotton plants.The emission of VOCs was significantly reduced with elevated N fertilization. No significant differences were found between the parasitisation rates by *C. marginiventris* on *S. exigua* larvae in the field cages in response to different rates of N-treatments. This suggests that, despite having significant N fertilization effects on herbivore-induced plant defenses, the parasitoids failed to differentiate between *S. exigua* larval feeding on physiologically different cotton plants that share huge constitutive volatile pools releasable when induced by herbivorous insects. To increase food security and to domesticate and utilize these crops' immense potential for sustainable agriculture, bioprospecting of VOCs in some crops including legumes is encouraged (Grebosz *et al.*, 2017).

4.2. Effects on phytohormone biosynthesis

Phytohormones play a crucial role in regulating the physiological and developmental activities of plants including leaf expansion, seed germination, stem elongation, flowering, and seed development (Jang *et al.*, 2008). Phytohormone production is significantly affected by N fertilization, production of some phytohormones is increased, whereas others are decreased. Jang *et al.* (2008) investigated the impact of different levels of N fertilizer in the form of urea (as described above) on two important endogenous phytohormones i.e., gibberellin (GA) and jasmonic acid (JA) in three different rice (*Oryza sativa*) cultivars (as described above). The biosynthesis of endogenous GA significantly increased in all three rice cultivars with elevated N levels but the rate of increase differed among different cultivars. In cv. Daesanbyeo, the rate of increase of GA_{12} contents was 3.6 times at 36.8 kg/ha N (basic rate) and 6.1 times at 73.6 kg/ha N (double rate) as compared to the control. The content of shoot GA_1 was increased 2.4 and 4.4 fold at basic and double N application rate, respectively. All other GA contents also increased steadily with increasing N application. In cv. Dongjinbyeo, both GA1 and GA12 contents increased more than 3 times in response to increased N application as compared to control. Other GAs also showed similar results. In cv. Junambyeo, the rate of increase of GA_{12} contents was 3.7 and 7.5 folds at basic and double N levels, respectively compared to the control. N application also increased the contents of GA_8, GA_{12}, GA_{19}, GA_{20}, and GA_{53}. When all three rice cultivars are taken into account, the most abundant endogenous GA type found was GA_{19} followed by GA_{53} and GA_{12}. The GA_1 contents increased significantly in all the rice cultivars with elevated N application rates. On the other hand, JA contents significantly increased with the basic N application but decreased with the double N application. The above results indicate that elevated N levels affect the biosynthesis of GA and JA differently in rice.

Another study (Chen *et al.*, 2008b) assessed the impact of different levels of N-fertilization (42, 112, 196, and 280 ppm) on the emission rate of JA and SA phytohormones from leaves of cotton plants (*Gossypium hirsutum*) in response to beet armyworm (*S. exigua*) feeding damage. Although there was a higher concentration of JA in herbivore-induced leaves compared to undamaged leaves, with increasing N levels, there was a sharp and significant decrease in JA accumulation in herbivore-damaged leaves. After 48 hours of feeding, herbivore-induced local leaves treated with 42 ppm N showed an 11-times increase in JA contents while the leaves with 196 ppm treatment showed only 1.5-fold increase. On the other hand, herbivore-induced local leaves treated with 112ppm N displayed a significant decrease in SA concentration and the leaves with 196 ppm treatment showed the lowest concentration of SA. Like local leaves, systemic leaves treated with the least N displayed the highest JA concentrations, whereas those leaves treated with increasing N levels displayed decreased SA concentrations.

4.3. Effects on plant defense

In order to combat herbivores and pathogens, plants perform both indirect and direct defenses (Olson *et al.*, 2009). Olson *et al.* (2009) conducted two sets of dual-choice wind tunnel experiments to find out the effect of N levels on parasitoid attraction. The aim of the first set experiment was to investigate the effect of N level on stimulation of volatile emission. In this regard, undamaged plants were tested against induced plants (herbivore damaged), the former receiving normal N dose (1 gm) and the latter receiving various levels of N (0 gm, 0.5 gm, 1 gm, and 2 gm N). Although the effect of N was not perceptible in this experiment, in reality, female parasitoids were able to distinguish between these undamaged and damaged plants no matter what the N levels received by the plants during the period of their growth. The above results demonstrate that the N levels in the soil tested do not hamper the production of herbivore-induced volatiles in cotton plants. The aim of the second set of experiments was to determine the impact of N levels on the release of herbivore-induced volatiles. In order to do this, one group of induced plants treated with a normal (recommended) dose of N (1gm) was tested against another group of induced plants treated with different doses of N (0 gm, 0.5 gm, and 2 gm). The female parasitoids preferred the induced plants treated with a recommended dose of N significantly more frequently than the induced plants treated with 0 gm and 2 gm N, but they did not make a significant choice between the recommended dose and 0.5 gm N. It indicates that although no obstruction of the emission of herbivore-induced volatiles was made by N dose levels, alteration of either quality or quantity of volatile compounds occurred. Results from the chemical analysis showed that there were differences in the concentrations of volatiles emitted from induced cotton plants treated with a recommended dose level of N (1 gm) and plants treated with 0 gm N or 2 gm N, the normal level having much higher than the other doses. Yet, N level highly influences the release of induced volatile and wasp responses; for improving the recruitment of natural enemies by herbivore-induced cotton plants, the recommended N level or lower than the recommended level for example 0.5 gm used in the experiment would likely be preferred over higher than the recommended N levels.

To understand the direct defense of cotton plants, dual choice experiments were carried out releasing *S. exigua* larvae on undamaged and induced cotton leaves (grown under 0.0 gm, 1.0 gm, and 2.0 gm) in Petri dish. Undamaged plants were more preferred than induced plants across all N levels applied to cotton plants during their growth (0.0 gm undamaged against 0.0 gm induced, 1.0 gm undamaged against 1.0 gm induced, and 2.0 gm undamaged against 2.0 gm induced, respectively). It demonstrates that the ability of herbivore-induced plants to discourage larval feeding was not hindered by the N level.

4.4. Effects on hemolymph protein profile of herbivore

While parasitoids feed on herbivorous insects, they consume hemolymph from the tissue of insects. For instance, the larval parasitoid, *C. marginiventris* feeds on the hemolymph of *S. exigua* larvae which might affect their development (Chen *et al.,* 2014). Although the total hemolymph contents of *S. exigua* are not affected by the N application rate, the total concentration of protein in *S. exigua* larvae are known to decrease after being parasitized by *C. marginiventris.* Hence, the decreased total protein concentrations in parasitized *S. exigua* larvae reflect the feeding of proteins by *C. marginiventris.*

No difference in protein concentration was observed in *S. exigua* larvae feeding on host plants of various N treatments. Two proteins were known to dominate in the larval hemolymph of *S. exigua* with a molecular weight of ca. 84 and 170 kDa, respectively. No significant effect of 170 kDa proteins was reported in *S. exigua*. So, this protein was not involved in slowing down the development of *C. marginiventris* treated with low N. On the contrary, when the unparasitized larvae were taken into account at 42 ppm N-treatment, the 84 kDa protein concentrations were significantly decreased. It indicates that the 84 kDa protein is significantly affected by food N. Nevertheless, in the larval parasitoid of *C. marginiventris* no significant differences were observed in the concentrations of 84 kDa protein across N treatments. In all the treatments more than 42 ppm, the N concentration of proteins was reduced by ½ to 2/3-folds in parasitized compared with unparasitized host larvae. In this way, reductions of proteins due to parasitism were found at high N treatment which occurred directly or indirectly by the modification of the development of the host. On the other hand, in parasitized larvae, the level of 84 kDa protein at 42 ppm treatment was increased twofold compared with unparasitized larvae. These results were explained by several factors. First, this difference in protein concentration was due to developmental delays caused by the host through parasitism. Secondly, a shift in ratios of nutrients for the long-term development of *C. marginiventris,* for instance, the ratio of protein to digestible carbohydrates (P: C) in food plants. Another possible explanation for delayed development of *C. marginiventris* larvae might be alterations in the relative level of allelochemicals in *S. exigua* larval hemolymph feeding on cotton plants with low N fertilization (Chen *et al.,* 2014).

5. Conclusions

It has been estimated that about half of all people on Earth are today nourished as a result of the usage of synthetic N fertilizer. Nitrogen is essential for plant growth at the optimal rate but both extremely low and high amount of nitrogen exerts harmful effects on the plant-herbivore-natural enemy tri-trophic system. The impact of N-fertilization is highly variable and does not correlate with N application rates. To date, the ecological and economic consequences of variable N-fertilization are less understood, mostly in field conditions. Therefore, the following actions could be recommended for future research: (i) explore the underlying mechanism by which variable N-fertilization modifies plant species richness, the abundance of insect pests, and their larval feeding and growth; (ii) conducting more research on the development of parasitoids and predatory insects are affected by elevated N-fertilization; (iii) investigation of emission and mechanism of action of VOCs and phytohormones in relation to variable N-fertilization. To sum up, controlled and optimal application of N-fertilizers is necessary for maintaining balance in a crop-herbivore-natural enemy tri-trophic system.

Data availability

Not applicable.

Conflict of interest

None to declare.

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