Green stink bug,*Nezaraviridula*(Hemiptera: Pentatomidae)an emerging insect pest of maize in India

Comment [A1]: Proposal :
First report of Nezaraviridula (Hemiptera: Pentatomidae) an emerging insect pest of maize in India

Abstract

The green stink bug *Nezaraviridula* (Linnaeus) (Hemiptera: Pentatomidae)is aneconomic insect pest of many cultivated plants around the world.Recently, high population of green stink bug were observedin maize crop during March–May 2020 in Bihar, a leading state of maize production in India. The population dynamics, damage symptoms, morpho-variants of *N. viridula*on maize were studied during 2020 and 2021 and presented in this report.The adults of *N. viridula* were first recorded in maize field during third week of March 2020 and in the same period of year 2021. The mean maximum adults' population of *N. viridula*was 43adults/ $m^2$and 34.33 adults/ m^2 observed during April 2020 and 202, respectively onmaize crop. The high population densities of thepest may be due to the migration of bugs from other field crops and native vegetationto the cultivatedmaize crop. During the course of the investigation,maximum 18 adult bugs per plant and 13 adult bugs per corn were recorded during fourth week of April 2020, whereas, maximum 12 adults per plant and 8 adults per cob were recorded during April 2021.The bugs were damage standing maize plants and corn by sucking saps and hindering the growth of plants, cobs as well as the developing kernels. Consideringthe high population densities, regular appearance of the pest anddamage caused to maize crop in thestate created an alarming situation for thepest management researchers of the country todevelop suitable integrated pest management strategiesfor the management of *N. viridula* on maize in the near future.

Comment [A2]: The study of population changes, not population dynamics

Comment [A3]: It is mostly about the results, it does not refer to the study method. It is better to describe the study method in short.

Key words: Green vegetable bug, corn, *Zea mays*, Stink bug

INTRODUCTION

Green stink bug, *Nezaraviridula*is a polyphagous and widely distributed insect pest of agriculture throughout the world (Tood, 1989; Rea *et al*., 2002). More than 150 host plant species have been reported for *N. viridula*from different part of the world(Panizzi, 2008). It attacks mainly fruit trees and vegetables, but also reported from field crops and some ornamental plants and weeds (Tood, 1989; Ciceoi*et al.*, 2017). In India,*N. viridula* is an endemic insect pest on many crops, but neither reported on maize for its economic damage nor listed as insect pest of maize at national level or regional level by National Centre for Integrated Pest Management, New Delhi, India (Kumar *et al.*, 2014).

Stink bugs damage maize crops at various stages (Pinero and Miller, 2017). At early stages, severe infestations and feeding by *N. viridula*may deform the maize cob and stops cob development. High infestation at maturityof crops, especially near the tip of the cob showed shrunken and missing kernels (Leskey*et al*., 2012). The shrivelled kernels are more susceptible to fungal diseases (Ciceoi*et al*.,2017). The damage level due to infestation of stink bug varies from 3 % to 100% depending on crop varieties and type of maize (Leskey*et al*.,2012; Opoku*et al*.,2019).The economic threshold level of *N. viridula* on maize has not been found in literature; whereas Cissel*et al.* (2015) suggested one brown marmorated stink bug (*Halyomorphahalys*Stål)per cob is enough to cause significant quality reductions in maize. Nevertheless, one stink bug per two plants has

been recommended by Koch *et al.* (2017) as economic threshold level for stink bugs (Pentatomidae)on maize regardless of the species.

Bihar is an agrarian state located in the eastern region of India between latitude 24°20'10" N to 27°31'15" N and longitude 83°1950" E to 88°17'40" E. Bihar is one of the leading producers of maize in the country with production of 2.42 million tonnes of maize grain with area of 0.67 million ha during 2018-19 (DES, 2018). Previously, the stink bug, *N. viridula*is occasionally seen on maize field. In the present study, during the March, 2020,exceptionally large numbers of adults with few nympswere observed on maize causing severe damage to the maize plants especially cobs.Thisregion is strategically important with respect to maize production in the country due to high productivity and good quality of maize grain. Keeping in mind, the emerging potentiality of the region on maize productionand the threat of the stink bug, we describethe population dynamics, damage symptoms, morpho-variants and impact of infestation of *N. viridula*on post harvest storageof maize in Bihar,an eastern state of India.

Comment [A4]: The study of population changes, not population dynamics

MATERIALS AND METHODS

Comment [A5]: How did you do it, direct observation, netting, framing and direct counting.... In what way??
Materials and methods must be detailed.

The weekly population of *N. viridula* was recorded from first week of March 2020and 2021 to till harvest of maize crop during May 2020 and 2021 in the experimental farm of Veer Kunwar Singh College of Agriculture, Dumraon, Buxar, Bihar (latitude 25.55^{o} N, longitude 84.15° Eand elevation 61m above mean sea level). The 1 m^{2}area (12 plants)at three different spot of maize field was selected randomly every week and counted the stink bug population. Level of plant and cob infestations by *N. viridula*were also recorded and presented in percent infestation at weekly interval. During observations, highest number of stink bug on one plant as well as one cob was also recorded. Damage symptoms on green cobs as well as on dried mature cobs were also recorded. The mature dried cobs were kept under observations for six months to record development of any fungal infection as well as any other stored grain insect pests by observing the stored cobs on monthly interval.

Comment [A6]: Write the variety of corn grown
Explain the farm conditions
Tell how you chose the three points

Comment [A7]: Write how to calculate the percentage of pollution

Comment [A8]: Why six months?
Did you control the shape?
Seed health tests are done in two ways, direct and indirect. What method did you use?

Comment [A9]: Do we have a pest that enters the warehouse from the field regarding corn?
What is the relationship between another pest attack and thisGreen stink bug?

RESULTS

Damage symptoms

Direct damage of *N. viridula*was observed with feeding of adults at different immature part of the maize plants (Figure 1). Whereas, the symptoms of damage were seen with the spot of honey dew excretion during feeding by *N. viridula*at different parts of maize plants (Figure 2).Post feeding damage lesions were also observed on maize cob and kernels (Figure 3).Results of the study revealed that the mature cobs infested with *N. viridula*were susceptible to stored grain insect pest. The infestation of stored grain insect pest was initiated at damaged kernel by *N. viridula* (Figure 2e)

Comment [A10]: How did you get this sensitivity?

Level of infestation

The highest maize plant infestations of 83.33 per cent and 88.89 per cent by *N. viridula* were recorded during third week of April 2020 and 2021 (Table 1). During this period, maximum no. of 18 adults and 12 adults*of N. viridula* on single plant was recorded during 2020 and 2021, respectively. Maximum 91.48 percent and 77.78 per cent infestation of cobs by *N. viridula* was recorded during year 2020 and 2021, respectively. Maximum numbers of adults *i.e.* 13 adults and 8 adults of *N. viridula*were recorded on single cob during observations in both the years.

Comment [A11]: How did you get the percentages? There is nothing in the materials and methods?

Comment [A12]: How did you get the percentage of contamination?

Morpho- variants of *N. viridula*

Morphological variations were also recorded in the adult stages of *N. viridula*. Six morpho- variants were recorded. Among six morpho-variants, three were from basic morpho-variants *i.e.*entirely green, green with anterior margin of the pronotum and head yellow and entirely yellow (Figure 4). Others were derived from these three basic types. Mating between different morpho-variants were recorded and found that they were compatible to mate between different morpho-variants (Figure 5).

Population dynamics of *N. viridula*

During first week of March 2020 and 2021, *N. viridula* population in the maize field was very low. Whereas, increase in populations were recorded during last week of March 2020 and 2021 with 1.33 bugs/ m^2 and 1.67 bugs/ m^2. Population of *N. viridula*attained peak during the last week of April 2020 and third week of April 2021 with 43 bugs/ m^2 and 34.33 bugs/ m^2, respectively. Thereafter, decrease in population was recorded and disappear during May 2020 and 2021with harvest of maize crops (Figure 6).

DISCUSSION

Adults of stink bugsentered into the maize field to begin feeding on plant and sooner or later started feeding on maize cobs. This feeding habit of stink bugs on maize can result in economic damage (Clower, 1958; Ni *et al*.,2010). In the present study, adults of *N. viridula* appear in the maize field during third week of March 2020 and 2021, when maize plants in the area entre into the reproductive phase.Weekly observations revealed the sudden increase in the population of *N. viridula* in maize field during April 2020 and 2021 and recorded maximum of 18 adult bugs and 13 adult bugs on single maize plant and 13 adult bugs and 8 adult bugs on single maize cobalong with maximum 43 bugs population /m^2 and 34.33 bugs/ m^2 respectively in two years.Ciceoi*et al.*(2017)reporteda maximum of seven stink bugs adults/cob and 16 adults/plant (12 *H. halys*and 4 *N. viridula*) during cob formation and grain filling phases until the wax maturity in the maize field of Bucharest, Romania.The high population density of *N. viridula* may cause the significant yield loss as Cissel*et al.* (2015) suggested that one stink bug per plant is enough to cause maize damages, while Koch *et al.* (2017) suggested 1 stink bug/2 plants as economic threshold. The population of stink bugs recorded were very high in the present study for yield loss in maize as per observations of Koch *et al.* (2017) and Cissel*et.al*. (2015)as we did not quantified the yield loss in the present study.No eggs and nymphs of *N. viridula* were recorded from the maize field under observations. Whereas, mating between pairs of adults were recorded in the field including between different morphological variants. Six morpho-variants of *N. viridula* were recorded in the present study. Polymorphism with colour variations is very common in *N.viridula.* Earlier 10 different colour morphs which are derived from four basic types are reported by different researchers from various region of the world (Kiritaniand Yukawa, 1963; Yukawa andKiritani, 1965;Kiritani, 1970; VivianandPanizzi, 2002).The timing of infestations during mid to late March in maize, suggests that the majority of *N. viridula* adults were offspring of the generation earlier in the season which developed on other unknown host plants in the area. The sudden increase of the population of *N. viridula* on maize may be due to unavailability of suitable host plant in the area due to start of harsh summer season leads the migration of *N. viridula.* Shifting of stink bug populations to a suitable host due to unavailability of native vegetation is also reported earlier by Venugopal*et al.* (2014). Shifting of stink bugs result from their movement in an area in response to deteriorating suitability of host crops or native vegetation to the target crop plants (Todd, 1989). In Bihar state, wheat and maize along with seasonal vegetables like tomato, potato and brinjal is the major crops in *Kharif*(winter season) season in the area. Most of the winter crops have beenharvestedin the area from the field during end of March leads the shifting of stink bug

Comment [A13]: These results are based on which method?
How did you recognize the different morphs?
Aren't the different colors related to the amount of nutrition?

Comment [A14]: How do you know that the rest are derived from these three basic morphs?
What was the method of this work?

Comment [A15]: Do you know the definition of population dynamics?
These are population changes, not dynamics!!!

Comment [A16]: In biology, polymorphism is the occurrence of two or more clearly different morphs or forms, also referred to as alternative phenotypes, in the population of a species. To be classified as such, morphs must occupy the same habitat at the same time and belong to a panmictic population (one with random mating).
When individual colour variation is discontinuous within a species, that species is said to be polychromatic.

Comment [A17]: Coloration changes occur not only in individuals but in populations as well. These latter result from evolutionary pressures—i.e., agents of natural selection—that act upon the natural variations in colour types (morphs) found among the population.

adults to maize filed. Adults of *N. viridula* were observed aggressively feeding on the maize plant and cobs causing brown spots on the site of feeding on the green maize cobs. Infestation of stink bugs at the time of grain filling causes cloudy or white spot on the grain which continued till the harvest of mature cobs. We also observed that the early infestation of *N. viridula* on maize ear causes ovule abortion resulted in undeveloped maize grain. Earlier similar damage on maize by *N. viridula*was recorded by different researchers. During the early stages, before the grain fill, severeinfestations and feeding may deform the maize cob,making it look like a cow horn or boomerang, andexpose the kernels to bird and insect damages becausethe cob shuck also stops developing (Ciceoi*et al.*2017). At later stages,feeding is associated with a 'mottled' appearance,due to kernels scarring or bruising, especially nearthe tip of the ear, and shrunken and missing kernels (Leskey*et al.* 2012).High population of *N. viridula*during reproductive phase in maize field resulted in 83.33 per cent and 88.89 per cent infested plants and 91.48 per cent and 77.78 per cent infested cobs in two years cause significant damage to the maize crops. Earlier, Leskey*et al.* (2012) mentioned that sweet maize is a highly preferred host of stink bugs, with reported losses of 100%.Infestation of *N. viridula* on maize grain not only affects the quality of grain but also makes harvested cobs susceptible for stored grain insect pest. We observed the infestation of stored grain insect pest begins with the site of infestation mark on kernel by*N. viridula*.Although information on impact of *N. viridula* infestation on storage is not available in literature. Whereas, similar observation was also recorded by Ciceoi*et al.* (2017) that the stink bug damaged kernels are more susceptible to fungal diseases.

The high population of green stink bug, *N. viridula* in the maize field of Bihar suggests alarming situation for maize cultivation in the state as well as in the country as this insect not only causes quantitative losses but also qualitatively to maize grain, makes maize grain unsuitable for long term storage. The alarming situations of infestation in maize by green stink bug in Bihar, ecological study with respect to host plants of *N. viridula*as well as timing of maize planting which leads to the timely harvest of the crops in the end of March before shifting of *N. viridula*on maize.

Considering the recent high population and regular appearance of *N. viridula* from last two years along with significant damage to the maize crop in this region, there is a need for urgent investigations of the eco-biology, behaviour, spatial and temporal distribution, and response to management practices of this species, so that effective management strategies can be designed in the near future. Failing this, the maize cultivation in Bihar as well as in different state of India may suffer seriously from this bug.

Comment [A18]: How did you come to this conclusion? Did you design an experiment?

Comment [A19]: You can't say that for sure. Maybe bad years will not come to corn??

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Table 1. Level of infestation of *N. viridula* on maize plants and cobs during Year 2020 and 2021 at Dumraon, Bihar

Date of Observation	Number of plant observed	No of plant infested	Percent plant infestation	No of cob observed	Number of cob infested	Percent cob infestation
Year 2020						
21-03-2020	36	1	2.78	25	0	0
28-03-2020	36	4	11.11	35	1	2.86
04-04-2020	36	8	22.22	45	6	13.33
11-04-2020	36	15	41.67	52	20	38.46
18-04-2020	36	25	69.44	47	43	91.49
25-04-2020	36	30	83.33	55	48	87.27
02-05-2020	36	23	63.89	51	39	76.47
09-05-2020	36	2	5.56	53	2	3.77
Year 2021						
25-03-2021	36	3	8.33	20	3	15
01-04-2021	36	10	27.78	29	8	27.59
08-04-2021	36	25	69.44	47	30	63.83
15-04-2021	36	30	83.33	45	35	77.78
22-04-2021	36	32	88.89	49	33	67.35
29-04-2021	36	29	80.56	50	35	70
06-05-2021	36	17	47.22	48	20	41.67
13-05-2021	36	5	13.89	45	5	11.11

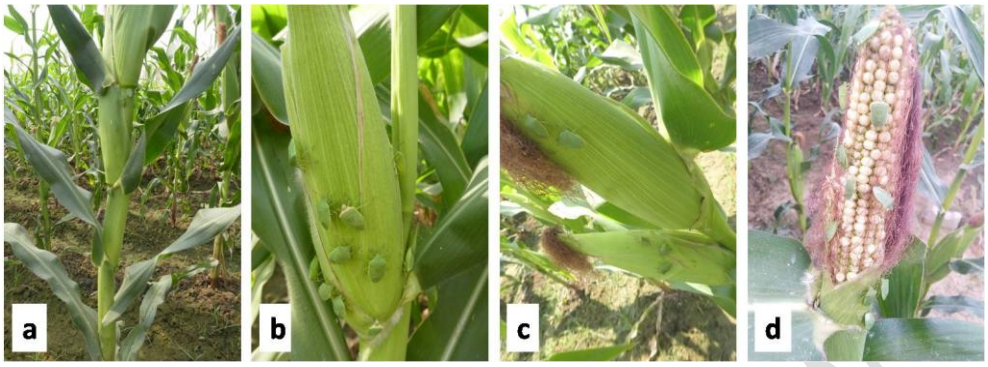

Figure 1. Infestation of *N. viridula* on maize plant. a) adult bugs feeding at plant nodal area, b) adult bugs feeding on basal part of the developing cob, c) adult bugs feeding on apical part of the developing cobs, and d) adult bugs feeding on an open developing cob

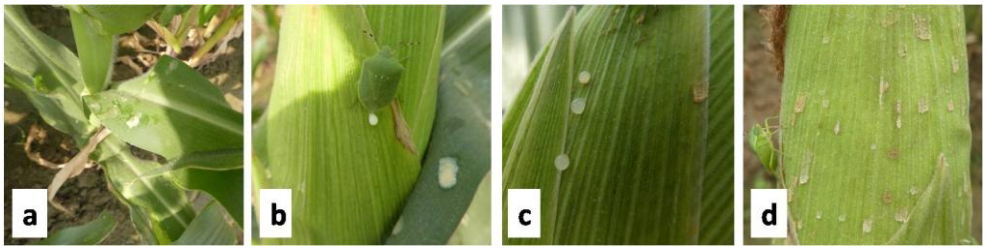

Figure 2. Excretion of honeydew by *N. viridula* on maize plant. a) Sign of honeydew excretion by bugs on leaf, b) adult bug excreting honeydew during feeding, c) excreted honeydew droplet on cob, and d) dried honeydew on cob

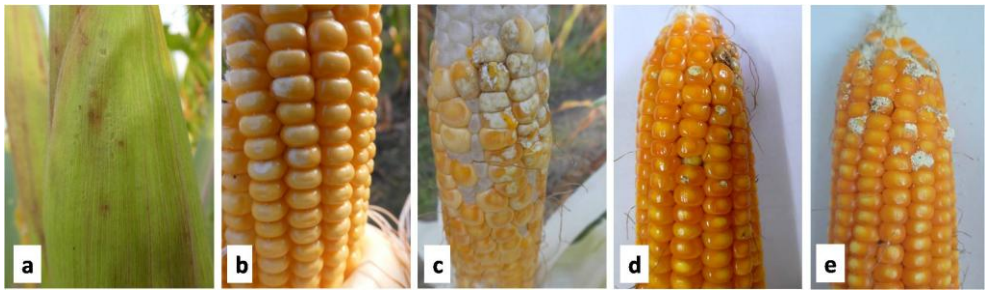

Figure 3. Damage symptoms of *N. viridula* on maize cob. a) brown spot on maize cob at the site of bug feeding b) bug feeding causes white/ cloudy spot on maize kernel, c) abortion, whitening, distortion of maize kernels due to excessive bug feeding, d) feeding damage sign on mature maize cob and e) infestation of stored grain insect pest on *N. viridula* infested mature cob

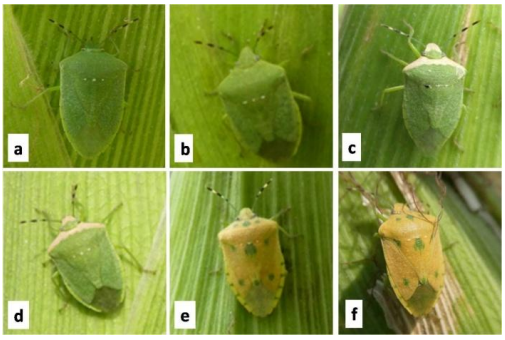

Figure 4. Morphotypes of *N. viridula* on maize plant. a) entirely green, b) entirely green with dark wing membrane, c) green with anterior margin of the pronotum and head yellow d),green with anterior margin of the pronotum and head yellow withdark wing membrane, e) entirely yellow, and f) entirely yellow with dark wing membrane

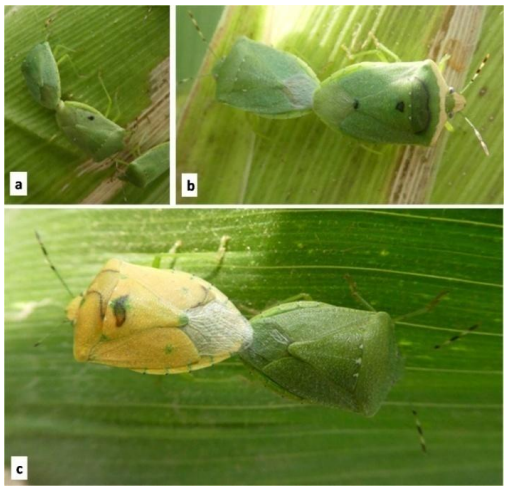

Figure 5. Mating between different morphotypes of *N. viridula*. a) mating between entirely green, b) mating between entirely green and green with anterior margin of the pronotum and head yellow and c) mating between entirely green and entirely yellow

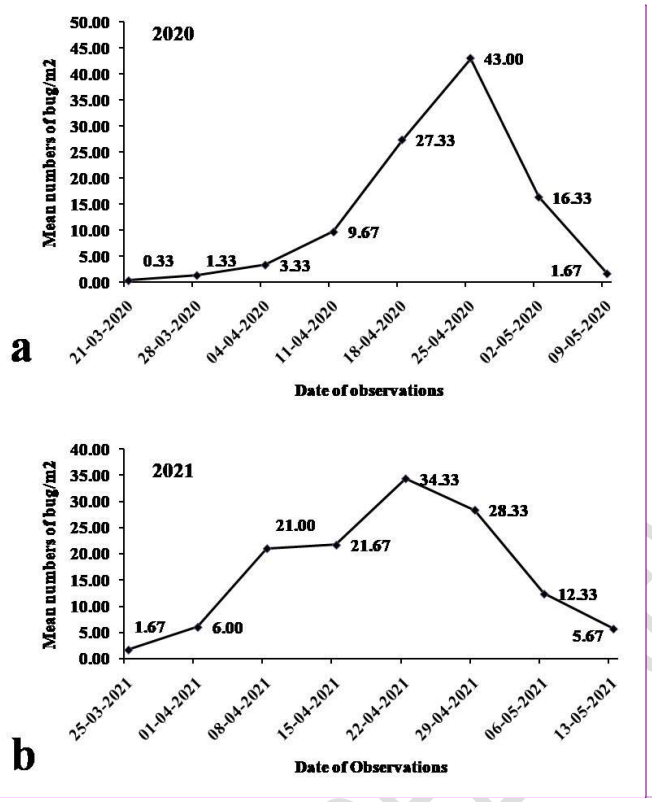

Figure 6. Population dynamics of *N. viridula* on maize. a) in the year 2020 and b) in the year 2021

Comment [A20]: How do you connect the two dots with a line when you only have statistics at one time? This is wrong. be corrected
Perhaps there will be demographic changes that are different between the two times.

Comment [A21]: ??